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<b>(54) Title:</b> CHIMERIC POLYPEPTIDES CONTAINING CHEMOKINE DOMAINS  <b>(57) Abstract</b>  This invention provides a chimeric DNA molecule comprising a sequence encoding a chemokine polypeptide covalently attached to a heterologous polypeptide, the encoded chimeric polypeptide, and uses thereof.		

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## CHIMERIC POLYPEPTIDES CONTAINING CHEMOKINE DOMAINS

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BACKGROUND OF THE INVENTION

The present invention relates generally to chimeric polypeptides containing chemokine polypeptide domains. More specifically, the invention relates to the expression in host cells of recombinant polynucleotide sequences encoding chemokine polypeptides covalently attached to heterologous polypeptides, and the use of such chimeric polypeptides as research tools for identifying chemokine receptors, as vaccine adjuvants, as agents for the chemotactic recruitment of migratory cells, as agents for the stimulation or inhibition of angiogenesis, as agents against autoimmune diseases and inflammation, and as agents to inhibit the binding of HIV to certain receptors.

Chemokines (or chemotactic cytokines) are a class of cytokine molecules capable of chemotactically attracting migratory cells, and are involved in cell recruitment and activation in inflammation. Chemokines generally have small molecular weights in the range of 8-10 kDa and, like other small proteins such as cytokines, are believed to be rapidly inactivated *in vivo*, resulting in relatively short biological half-lives for these proteins. Most chemokines can be divided into two subgroups, CXC or CC, on the basis of the spacing of two highly-conserved cysteine amino acids near the amino terminus of these proteins. Within the CXC and CC subgroups, chemokines are further grouped into related families based on amino acid sequence similarity between them. CXC chemokine families include the IP-10 and Mig family; the GRO $\alpha$ , GRO $\beta$ , and GRO $\gamma$  family; the interleukin-8 (IL-8) family; and the PF4 family. CC chemokine families include the monocyte chemoattractant protein (MCP) family; the family including macrophage

inhibitory protein-1 $\alpha$  (MIP-1 $\alpha$ ), macrophage inhibitory protein-1 $\beta$  (MIP-1 $\beta$ ), and regulated on activation normal T cell expressed (RANTES); and the lymphotactin family. The chemokines stromal cell-derived factor 1 $\alpha$  (SDF-1 $\alpha$ ) and stromal cell-derived factor 1 $\beta$  (SDF-1 $\beta$ ) form a chemokine family that is approximately equally related by amino acid sequence similarity to the CXC and CC chemokine subgroups. Individual members of the chemokine families are known to be bound by at least one chemokine receptor, with CXC chemokines generally bound by members of the CXCR class of receptors, and CC chemokines by members of the CCR class of receptors. For example, SDF-1 $\alpha$  is known to be a ligand for the CXCR receptor fusin/CXCR4, and MIP-1 $\alpha$  is bound by the CCR receptors CCR1, CCR4, and CCR5.

The presence of a chemokine gradient attracts migratory cells such as lymphocytes, leukocytes, and antigen-presenting cells (APCs) that may participate in autoimmune reactions, inflammation, or normal immune responses, or that may release other intercellular factors to stimulate or inhibit angiogenesis or other cellular processes. For example, the initiation of autoimmune disease requires the infiltration or recruitment of lymphocytes able to respond against self proteins into the organ bearing the antigenic self proteins. Inflammatory atherosclerotic lesions are due in part to infiltration of the vascular compartment by leukocytes recruited to the site. To induce an immune response, antigenic proteins and glycoproteins must bind to the surface of B lymphocytes to stimulate antibody production, and must be taken up by antigen-presenting cells, processed, and represented to T lymphocytes to mediate a T-lymphocyte response. Migratory cells that secrete IP10 or IL-8, when attracted by a chemokine gradient to a particular site, respectively may inhibit or stimulate the formation of blood vessels at that site. Chemokines may be used to establish a chemoattractive gradient for migratory cells that are expressing the appropriate chemokine receptors, or to obscure an existing chemoattractive gradient.

Chemokine receptors are also involved in functions other than chemotaxis, such as interacting with viral proteins. HIV-1 is known to bind to certain proteins on the surface of cells in order to gain entrance into these cells and replicate or integrate the viral gene into the host DNA. The CD4 protein on T lymphocytes and other cells, including certain antigen presenting cells, has been shown to be bound by the HIV-1 viral envelope protein gp120. This is believed to induce in gp120 a conformational change that then exposes

regions of gp120 and perhaps CD4 that subsequently bind to a chemokine receptor. To date CXCR4 (also known as fusin), CCR5, and several other chemokine receptors have been identified as co-receptors for HIV-1. Monocyte-tropic (M-tropic) isolates of HIV-1 require interaction with CCR5 in order to infect cells, while T-lymphocyte-tropic (T-tropic) HIV-1 isolates require another coreceptor, CXCR4, for infection. There is some evidence indicating that HIV-1 can also use other CCR receptors such as CCR2 and CCR3 to gain entry into cells expressing them. For some HIV-2 isolates, it appears that certain chemokine receptors such as fusin/CXCR4 alone can provide the cell-surface protein needed for binding and entrance into the cell.

10 HIV-1 infection of cells expressing CD4 and fusin/CXCR4 is greatly decreased by the addition of purified SDF-1 $\alpha$ , which is bound by fusin/CXCR4. We have found that preincubation of cells in the presence of purified SDF-1 $\alpha$  for a short period of time at 37°C causes a profound down-regulation of the receptor. This down-regulation of fusin/CXCR4 correlates with a decrease in the ability of HIV-1 to infect cells.

15 There is a continuing requirement for new compositions that will enhance, alter, or inhibit the effects of chemokine-receptor interactions, and for methods for their use.

#### SUMMARY OF THE INVENTION

Applicants have for the first time constructed novel chimeric DNA molecules encoding chimeric polypeptides comprising chemokine polypeptide domains. Chimeric polypeptides expressed from these constructs have exhibited novel properties, including novel interactions with cells expressing chemokine receptors.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide encoding a chimeric polypeptide, the chimeric polypeptide comprising at least one chemokine polypeptide covalently attached to at least one heterologous polypeptide. Preferably, the chemokine polypeptide is SDF-1 $\alpha$ , MIP-1 $\alpha$ , or MIP-1 $\beta$ , or is derived from SDF-1 $\alpha$ , MIP-1 $\alpha$ , or MIP-1 $\beta$ . Preferably, the heterologous polypeptide is an Fc polypeptide.

Another embodiment provides a composition comprising an isolated polynucleotide encoding a chimeric polypeptide, wherein a heterologous polypeptide is

covalently attached to the amino terminus of a chemokine polypeptide, preferably by a linker polypeptide.

Another embodiment provides a composition comprising an isolated polynucleotide encoding a chimeric polypeptide, wherein a heterologous polypeptide is covalently attached to the carboxyl terminus of a chemokine polypeptide, preferably by a linker polypeptide.

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide encoding a chimeric polypeptide, wherein the polynucleotide is selected from the group consisting of:

- 10 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 12 to nucleotide 1213;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 69 to nucleotide 1213;
- (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 72 to nucleotide 1213;
- 15 (d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 75 to nucleotide 1213;
- (e) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:2;
- 20 (f) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone S1-3 deposited under accession number ATCC 98338;
- (g) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone S1-3 deposited under accession number ATCC 98338;
- 25 (h) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:1;
- (i) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:1 from amino acid 20 to amino acid 328;
- 30 (j) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:1 from amino acid 22 to amino acid 328;

(k) a polynucleotide encoding a chimeric polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:1;

(l) a polynucleotide comprising a nucleotide sequence complementary to any one of the polynucleotides specified in (a)-(k) above; and

5 (m) a polynucleotide capable of simultaneously hybridizing under stringent conditions to sequences encoding the chemokine polypeptide and to sequences encoding the heterologous polypeptide in any one of the polynucleotides specified in (a)-(l) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID  
10 NO:2 from nucleotide 12 to nucleotide 1213; the nucleotide sequence of the full-length protein-coding sequence of clone S1-3 deposited under accession number ATCC 98338; or the nucleotide sequence of the mature protein-coding sequence of clone S1-3 deposited under accession number ATCC 98338.

In a further embodiment, the present invention provides a composition comprising  
15 an isolated polynucleotide encoding a chimeric polypeptide, wherein the polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:4 from nucleotide 12 to nucleotide 1207;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID  
20 NO:4 from nucleotide 69 to nucleotide 1207;

(c) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:4;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone SK2-2 deposited under accession  
25 number ATCC 98339;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone SK2-2 deposited under accession number ATCC 98339;

(f) a polynucleotide encoding a chimeric polypeptide comprising the  
30 amino acid sequence of SEQ ID NO:3;

(g) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:3 from amino acid 20 to amino acid 326;

(h) a polynucleotide encoding a chimeric polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:3;

5 (i) a polynucleotide comprising a nucleotide sequence complementary to any one of the polynucleotides specified in (a)-(h) above; and

(j) a polynucleotide capable of simultaneously hybridizing under stringent conditions to sequences encoding the chemokine polypeptide and to sequences encoding the heterologous polypeptide in any one of the polynucleotides specified in (a)-(i) above.

10 Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:4 from nucleotide 12 to nucleotide 1207; the nucleotide sequence of the full-length protein-coding sequence of clone SK2-2 deposited under accession number ATCC 98339; or the nucleotide sequence of the mature protein-coding sequence of clone SK2-2 deposited  
15 under accession number ATCC 98339.

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide encoding a chimeric polypeptide, wherein the polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID  
20 NO:6 from nucleotide 15 to nucleotide 1225;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:6 from nucleotide 81 to nucleotide 1225;

(c) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:6;

25 (d) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone MP-1 deposited under accession number ATCC 98341;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone MP-2 deposited under accession  
30 number ATCC 98342;



(f) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone MP-6 deposited under accession number ATCC 98340;

5 (g) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone MP-1 deposited under accession number ATCC 98341;

(h) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone MP-2 deposited under accession number ATCC 98342;

10 (i) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone MP-6 deposited under accession number ATCC 98340;

(j) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:5;

15 (k) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:5 from amino acid 23 to amino acid 331;

(l) a polynucleotide encoding a chimeric polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:5;

20 (m) a polynucleotide comprising a nucleotide sequence complementary to any one of the polynucleotides specified in (a)-(l) above; and

(n) a polynucleotide capable of simultaneously hybridizing under stringent conditions to sequences encoding the chemokine polypeptide and to sequences encoding the heterologous polypeptide in any one of the polynucleotides specified in (a)-(m) above.

25 Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:6 from nucleotide 15 to nucleotide 1225; the nucleotide sequence of the full-length protein-coding sequence of clones MP-1, MP-2, and MP-6 deposited under accession numbers ATCC 98341, ATCC 98342, and ATCC 98340, respectively; or the nucleotide sequence of the mature protein-coding sequence of clones MP-1, MP-2, and MP-6  
30 deposited under accession numbers ATCC 98341, ATCC 98342, and ATCC 98340, respectively.

In a further embodiment, the present invention provides a composition comprising an isolated polynucleotide encoding a chimeric polypeptide, wherein the polynucleotide is selected from the group consisting of:

- 5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 16 to nucleotide 1226;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 85 to nucleotide 1226;
- (c) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:8;
- 10 (d) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone MPB-X deposited under accession number ATCC XXXXX;
- (e) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone MPB-X deposited under accession number  
15 ATCC XXXXX;
- (f) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:7;
- (g) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:7 from amino acid 24 to amino acid 331;
- 20 (h) a polynucleotide encoding a chimeric polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:7;
- (i) a polynucleotide comprising a nucleotide sequence complementary to any one of the polynucleotides specified in (a)-(h) above; and
- (j) a polynucleotide capable of simultaneously hybridizing under  
25 stringent conditions to sequences encoding the chemokine polypeptide and to sequences encoding the heterologous polypeptide in any one of the polynucleotides specified in (a)-(i) above.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:8 from nucleotide 16 to nucleotide 1226; the nucleotide sequence of the full-length  
30 protein-coding sequence of clone MPB-X deposited under accession number ATCC

XXXXXX; or the nucleotide sequence of the mature protein-coding sequence of clone MPB-X deposited under accession number ATCC XXXXXX.

In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, preferably a mammalian cell, transformed with such polynucleotide compositions.

Processes are also provided for producing a chimeric polypeptide, which comprise:

- (a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium; and
- (b) purifying the protein from the culture.

The polypeptide produced according to such methods is also provided by the present invention. Preferred embodiments include those in which the polypeptide produced by such process is a mature form of the polypeptide.

In other embodiments, the present invention provides a composition comprising a chimeric polypeptide, the chimeric polypeptide comprising at least one chemokine polypeptide covalently attached to at least one heterologous polypeptide. Preferably, the chemokine polypeptide is SDF-1 $\alpha$ , MIP-1 $\alpha$ , or MIP-1 $\beta$ , or is derived from SDF-1 $\alpha$ , MIP-1 $\alpha$ , or MIP-1 $\beta$ . Preferably, the heterologous polypeptide is an Fc polypeptide.

A further embodiment provides a composition comprising a chimeric polypeptide, wherein a heterologous polypeptide is covalently attached to the amino terminus of a chemokine polypeptide, preferably by a linker polypeptide.

Another embodiment provides a composition comprising a chimeric polypeptide, wherein a heterologous polypeptide is covalently attached to the carboxyl terminus of a chemokine polypeptide, preferably by a linker polypeptide.

In another embodiment, the present invention provides a composition comprising a chimeric polypeptide, wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:1;
- (b) the amino acid sequence of SEQ ID NO:1 from amino acid 20 to amino acid 328;

(c) the amino acid sequence of SEQ ID NO:1 from amino acid 21 to amino acid 328;

(d) the amino acid sequence of SEQ ID NO:1 from amino acid 22 to amino acid 328; and

5 (e) fragments of the amino acid sequence of SEQ ID NO:1.

Preferably, such chimeric polypeptide comprises the amino acid sequence of SEQ ID NO:1.

In a further embodiment, the present invention provides a composition comprising a chimeric polypeptide, wherein the chimeric polypeptide comprises an amino acid  
10 sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:3;

(b) the amino acid sequence of SEQ ID NO:3 from amino acid 20 to amino acid 326; and

(c) fragments of the amino acid sequence of SEQ ID NO:3.

15 Preferably, such chimeric polypeptide comprises the amino acid sequence of SEQ ID NO:3.

In another embodiment, the present invention provides a composition comprising a chimeric polypeptide, wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of:

20 (a) the amino acid sequence of SEQ ID NO:5;

(b) the amino acid sequence of SEQ ID NO:5 from amino acid 23 to amino acid 331; and

(c) fragments of the amino acid sequence of SEQ ID NO:5.

25 Preferably, such chimeric polypeptide comprises the amino acid sequence of SEQ ID NO:5.

In a further embodiment, the present invention provides a composition comprising a chimeric polypeptide, wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:7;

30 (b) the amino acid sequence of SEQ ID NO:7 from amino acid 24 to amino acid 331; and

(c) fragments of the amino acid sequence of SEQ ID NO:7.

Preferably, such chimeric polypeptide comprises the amino acid sequence of SEQ ID NO:7.

Polypeptide compositions of the present invention may further comprise a  
5 pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such polypeptide are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical  
condition which comprises administering a therapeutically effective amount of a  
composition comprising a polypeptide of the present invention and a pharmaceutically  
10 acceptable carrier.

The present invention also provides methods for identifying molecules capable  
of interacting with a chimeric polypeptide which comprise:

(a) combining a composition comprising a chimeric polypeptide, the  
chimeric polypeptide comprising at least one chemokine polypeptide covalently  
15 attached to at least one heterologous polypeptide, with a composition comprising  
molecules to be tested for interaction, forming a first mixture;

(b) combining the first mixture with a composition comprising  
indicator molecules, so that the indicator molecules are capable of being altered  
by the first mixture; and

20 (c) detecting the presence of altered indicator molecules.

Methods are also provided for attracting migratory cells to a region of an  
organism which comprises administering therapeutically effective amounts of at least one  
composition comprising a chimeric polypeptide.

Methods for stimulating or inhibiting angiogenesis, which comprise administering  
25 therapeutically effective amounts of at least one composition comprising a chimeric  
polypeptide, are also provided.

Methods are also provided for preventing, treating, or ameliorating an  
inflammatory or an autoimmune condition, which comprise administering therapeutically  
effective amounts of at least one composition comprising a chimeric polypeptide.

30 Methods for enhancing antigen-presenting cell activity, which comprise  
administering therapeutically effective amounts of at least one composition comprising a

chimeric polypeptide, wherein at least one chimeric polypeptide comprises antigen molecules, are also provided.

Methods are provided for inducing an immune response which comprise administering a vaccine and therapeutically effective amounts of at least one composition comprising a chimeric polypeptide.

Methods for altering receptor function which comprise causing a receptor to bind at least one chimeric polypeptide, and for decreasing receptor function which comprise causing a receptor to bind at least one chimeric polypeptide, resulting in a decrease in the number of functional receptor molecules, are provided.

Methods are provided for preventing, treating, or ameliorating HIV infection which comprise administering therapeutically effective amounts of at least one composition comprising a chimeric polypeptide. Preferably, the chemokine polypeptide of the chimeric polypeptide comprises SDF-1 $\alpha$ , MIP-1 $\alpha$ , or MIP-1 $\beta$ .

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description of preferred embodiments thereof.

#### BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the expression of chimeric polypeptides, described in Example 2.

Fig. 2 shows chimeric SDF-1 $\alpha$  polypeptide binding to cells expressing the fusin/CXCR4 receptor, as described in Example 3.

#### DETAILED DESCRIPTION OF THE INVENTION

The present inventors have for the first time constructed novel chimeric polypeptides comprising a chemokine polypeptide covalently attached to a heterologous polypeptide. These chimeric polypeptides interact with chemokine receptors and have novel properties.

As used herein, "chemokine" includes all molecules with chemotactic activity or derived from molecules with chemotactic activity by any kind of alteration, addition, insertion, deletion, mutation, substitution, replacement, or modification. Chemotactic activity for a particular cell population is the direct or indirect stimulation of the directed orientation or movement of such cell population. Preferably, the cell population comprises

circulating blood cells, bone marrow stem cells. More preferably, the cell population may include monocytes, B cells, T cells, basophils, eosinophils, neutrophils, natural killer (NK) cells, and bone marrow stem cells. Most preferably, the cell population may include monocytes, T cells, basophils, and bone marrow stem cells. Preferably, the chemokine has the ability to directly stimulate directed movement of cells. Whether a particular polypeptide has chemotactic activity for a population of cells can be readily determined by employing the polypeptide in any known assay for cell chemotaxis. Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed. by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. by Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28); Taub et al., J. Clin. Invest. 95:1370-1376, 1995; Lind et al., APMIS 103:140-146, 1995; Muller et al., Eur. J. Immunol. 25: 1744-1748; Gruber et al., J. of Immunol. 152:5860-5867, 1994; Johnston et al., J. of Immunol. 153: 1762-1768, 1994; all of which are incorporated herein by reference.

As used herein, "covalently attached" means the attachment of molecules to each other by covalent chemical bonds, either directly or through a linker molecule that is itself covalently attached to said molecules.

As used herein, "heterologous polypeptides" include all polypeptides that can be covalently attached to a chemokine polypeptide, including without limitation chemokines, cytokines, immunoglobulins, antigens, antibody-binding tags such as His, Flag, or myc, lectin-binding domains, toxins, kinases, proteases, other enzymes, structural proteins; polypeptides derived from the foregoing by any form of alteration, addition, insertion, deletion, mutation, substitution, replacement, or modification; but excluding thioredoxin. For example, chemokine polypeptides can be attached through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the chemokine, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a chemokine-IgM fusion would generate a

decaivalent form of the chemokine. In addition, it is possible to create a multivalent form of a chimeric polypeptide by connecting the chimeric polypeptide through a P<sub>i</sub> linkage to the phosphatidyl inositol present in micellular preparations.

5       Fragments of chimeric chemokine polypeptides are also encompassed by the present invention. Preferably, such fragments retain the desired activity of the polypeptide or modify it to create a desired activity. Fragments of polypeptides may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, *Bio/Technology* 10, 773-778 (1992) and in R.S. McDowell, *et al.*, *J. Amer. Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. The  
10       polypeptides provided herein also include polypeptides characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the polypeptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the polypeptide sequences may include the alteration, addition, insertion,  
15       deletion, mutation, substitution, replacement, or modification of a selected amino acid residue in the coding sequence. As one example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. As another example, an additional amino acid may be added to the N-terminus of the polypeptide. Also, the amino acid sequence of the polypeptide may be altered using random  
20       mutation techniques. It is also possible to attach to polypeptides other moieties, including without limitation carbohydrates, lipids, or polyethylene glycol, or to remove or alter such moieties. Techniques for such alterations, additions, insertions, deletions, mutations, substitutions, replacements, or modifications are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584). Preferably, such alteration, addition, insertion, deletion,  
25       mutation, substitution, replacement, or modification retains the desired activity of the polypeptide or modifies it to create a desired activity.

Other fragments and derivatives of the sequences of polypeptides which would be expected to retain polypeptide activity and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the  
30       disclosures herein. Such modifications are believed to be encompassed by the present invention.



The present invention also provides both full-length and mature forms of chimeric chemokine polypeptides. The full-length form of such polypeptides is identified in the sequence listing by translation of the protein-coding region, excluding introns, of the nucleotide sequence of each disclosed construct. The mature form of such polypeptides may be obtained by expression of the disclosed full-length polynucleotide (preferably those deposited with ATCC) in a suitable mammalian cell, preferably CHO or COS cell, or other host cell. The sequence of the mature form of the polypeptide may also be determinable from the amino acid sequence of the full-length form.

Chimeric chemokine polypeptides including chemokine polypeptides that are species homologs of disclosed polypeptides are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species. The invention also encompasses allelic variants of the disclosed chemokine polypeptides or chemokine-encoding polynucleotides; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode polypeptides which are identical, homologous or related to that encoded by the polynucleotides.

The present invention also includes polynucleotides capable of hybridizing under stringent conditions, preferably highly stringent conditions, to polynucleotides described herein. Highly stringent conditions include, for example, 0.2xSSC at 65°C; stringent conditions include, for example, 4xSSC at 65°C or 50% formamide and 4xSSC at 42°C. Preferably, such hybridizing polynucleotides are at least 70% homologous by sequence identity (more preferably, at least 80% homologous; most preferably 90% or 95% homologous) with the polynucleotide of the present invention to which they hybridize.

#### Preferred Chimeric Polypeptides and Polynucleotides Encoding Them

Amino acid sequences of chimeric chemokine polypeptides are set forth below, along with the sequences of polynucleotides encoding them. In these chimeric polypeptides, the chemokine has been linked to an Fc polypeptide by a [Gly-Ser]<sub>3</sub> linker peptide. The polynucleotides encoding these chimeric polypeptides were derived from chemokine cDNA sequences and genomic Fc sequences, as described in Example 1 below.

The sequence of a polynucleotide encoding one such chimeric polypeptide including an SDF-1 $\alpha$  domain is set forth in SEQ ID NO:2, with the protein-coding sequence (including introns) extending from nucleotide 12 to 1213. This polynucleotide has been identified as S1-2 or S1-3, the DNA sequences of these two constructs appearing to be identical. The amino acid sequence of the chimeric polypeptide encoded by S1-2 and S1-3 is set forth in SEQ ID NO:1. The chimeric polypeptide encoded by S1-2 and S1-3 is 328 amino acids in length, with the mature polypeptide produced by cleavage of the secretory leader sequence beginning at amino acid 20, 21, or 22 of SEQ ID NO:1, depending on how the polypeptide is processed. The polynucleotide construct S1-3 was deposited with the American Type Culture Collection on February 28, 1997 and given the accession number ATCC 98338.

The sequence of a polynucleotide encoding another such chimeric polypeptide that includes a domain derived from SDF-1 $\alpha$  is set forth in SEQ ID NO:4, with the protein-coding sequence (including introns) extending from nucleotide 12 to 1207. This polynucleotide has been identified as SK2-2. The amino acid sequence of the chimeric polypeptide encoded by SK2-2 is set forth in SEQ ID NO:3. The chimeric polypeptide encoded by SK2-2 is 326 amino acids in length, with the mature polypeptide produced by cleavage of the secretory leader sequence beginning at amino acid 20 of SEQ ID NO:3. The polypeptide encoded by SK2-2 differs from that encoded by S1-2 and S1-3 in that two amino acids have been deleted from the SK2-2 sequence so that cleavage of the secretory leader sequence is predicted to always produce a product beginning at amino acid 20 of SEQ ID NO:3. The polynucleotide construct SK2-2 was deposited with the American Type Culture Collection on February 28, 1997 and given the accession number ATCC 98339.

The sequence of a polynucleotide encoding a chimeric polypeptide that includes an MIP-1 $\alpha$  domain is set forth in SEQ ID NO:6, with the protein-coding sequence (including introns) extending from nucleotide 15 to 1225. This polynucleotide is identified as MP-1. The DNA sequence of MP-1 has been determined, and while the DNA sequences of MP-2 and MP-6 are anticipated to be identical to that of MP-1, these clones may contain some PCR-generated DNA sequence alterations. The amino acid sequence of the chimeric polypeptide encoded by MP-1, and presumably encoded by MP-2 and MP-6, is set forth in SEQ ID NO:5. The chimeric polypeptide encoded by MP-1 is 331 amino acids in length,

with the mature polypeptide produced by cleavage of the secretory leader sequence beginning at amino acid 23 of SEQ ID NO:5. The polynucleotide constructs MP-1, MP-2, and MP-6 were deposited with the American Type Culture Collection on February 28, 1997 and given the accession numbers ATCC 98341, 98342, and 98340.

- 5           The sequence of a polynucleotide encoding a chimeric polypeptide that includes an MIP-1 $\beta$  domain is set forth in SEQ ID NO:8, with the protein-coding sequence (including introns) extending from nucleotide 16 to 1226. This polynucleotide is identified as MPB-X. The amino acid sequence of the chimeric polypeptide encoded by MPB-X is set forth in SEQ ID NO:7. The chimeric polypeptide encoded by MPB-X is predicted to be 331 amino acids in length, with the mature polypeptide produced by cleavage of the secretory leader sequence beginning at amino acid 24 of SEQ ID NO:7.
- 10

#### Expression and Purification of Chimeric Polypeptides

- The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.
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- 20

- A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.
- 25

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLabs (Beverly, MA), Pharmacia (Piscataway, NJ) and InVitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, CT).

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

#### Uses of Chimeric Polypeptides

Chimeric chemokine polypeptides can be used as tools for identifying cells expressing receptor for the chemokine, or for studying binding of chemokine to isolated receptor molecules. The construct when incubated with cells expressing a receptor for the chemokine will bind to these cells and can be indicated using a commercially available fluorescently tagged antibody, or other protein, able to bind to the heterologous polypeptide

domain, such as the Fc region of human immunoglobulin, of the chimeric polypeptide. This will indicate cells having a surface receptor for a given chemokine as well as the density of this receptor on the cell surface.

Interactions between chimeric chemokine polypeptides and chemokine receptors can also be detected directly by measuring changes in surface plasmon resonance using a Biacore sensor (Pharmacia). The chemokine receptor or the chimeric polypeptide can be covalently immobilized to different flow cells on the Biacore sensor chip as recommended by the manufacturer. Molecules to be tested for interaction are then injected across the flow cells and binding is detected as a change in resonance units, a reflection of the mass of protein bound to the sensor chip surface.

Other suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, edited by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, published by Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22); Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995; all of which are incorporated herein by reference.

Chimeric chemokine polypeptides can also be used as vaccine adjuvants. Proteins and glycoproteins injected to induce an immune response must bind to surface of B lymphocytes to stimulate antibody production and must be taken up by antigen presenting cells, processed, and represented to T lymphocytes to mediate a T lymphocyte response. By including with the antigen injection a chimeric chemokine-Fc polypeptide the infiltration of the necessary APCs and lymphocytes will be induced by the chemoattractive presence of the chemokine. One advantage of including an Fc domain in the chimeric polypeptide is that the chimeric polypeptide will have a longer biological half life than the chemokine alone would have. Also, by including in the chimeric polypeptide an Fc domain able to bind to existing Fc-receptors on cells at the site of injection, the chemokine activity will be concentrated at the site, much like a depot so that the chemokine gradient could be maintained over a long enough period of time to ensure the infiltration of the necessary responding cell populations.

Chimeric chemokine polypeptides can also be used to enhance the activity of antigen-presenting cells (APCs). The presence of the chemokine domain of the chimeric polypeptide would chemotactically attract APCs. Additionally, an antigenic molecule could be included in the chimeric polypeptide for delivery to the APC. When such an antigen-  
5 containing chimeric polypeptide binds to the surface of an APC and is internalized, and the chimeric polypeptide is degraded within the APC, the antigenic portion of the chimeric polypeptide would be freed for interaction with MHC proteins and presentation on the surface of the APC.

Chimeric chemokine polypeptides can also be used to affect the chemotactic  
10 recruitment of migratory cells. Chimeric chemokines may be used to establish a chemoattractive gradient for migratory cells that are expressing the appropriate chemokine receptors, or to obscure an existing chemoattractive gradient. By including a large or particularly stable heterologous polypeptide in the chimeric polypeptide, the chimeric polypeptide will have a longer biological half life and will be able to establish a longer  
15 lasting chemoattractive gradient, and will be more effective in obscuring a preexisting gradient. Also, a heterologous polypeptide domain may be selected that, by binding to particular molecules or cells, will target the chimeric chemokine polypeptide to a particular site in order to establish a chemoattractive gradient at that site. By altering chemoattractive gradients, chimeric chemokine polypeptides can be used to treat inflammatory and  
20 autoimmune disorders that require the recruitment of migratory cells. Also, by attracting to a particular site migratory cells that produce other intercellular factors such as IL-8 or IP-10, chimeric chemokine polypeptides may for example be used to stimulate angiogenesis at that site (if, for example, the recruited migratory cells were secreting IL-8) or to inhibit angiogenesis at that site (if, for example, the recruited migratory cells were secreting IP-10).  
25 In addition, by establishing a gradient of chimeric chemokine polypeptide within the bone marrow of a bone marrow transplant recipient, the chimeric chemokine polypeptide could be used to recruit the transplanted bone marrow cells to the bone marrow where they are needed. Similarly, other cellular processes could be affected by chimeric chemokine polypeptides, by using them to attract particular classes of migratory cells secreting  
30 determined factors.

Chimeric chemokine polypeptides can also be used to affect the nature of chemokine-receptor interactions, and may block the binding of endogenous molecules to their receptors. By binding to a receptor, chimeric chemokines may deliver a signal similar to that received via the normal ligand. When the heterologous polypeptide is an Fc polypeptide, because of its bivalent nature this signal may be delivered at a lower molar concentration of ligand. The signal delivered by binding the chimeric polypeptide may have some properties different from that of the normal ligand because of the structure of the chimeric polypeptide. This could include prolonged triggering/activation or decreased activation. The chimeric polypeptides, because of their larger size or the nature of the structure of the heterologous polypeptide domain, will have a longer half life in vivo compared to monomeric ligand, possibly leading to prolonged signaling/activation. Also the larger size of the chimeric polypeptide will cause some steric hindrance which may block the binding of the natural ligand. A chimeric chemokine polypeptide may desensitize a receptor's response to normal ligand by binding and inactivating further signaling through the same receptor. In the case where a receptor has more than one signaling function, the chimeric chemokine polypeptide may inhibit one form of signaling while enhancing or altering another. Also, a chimeric chemokine polypeptide may bind to a receptor and cause down regulation and /or internalization of the receptor. Additionally, a chimeric chemokine polypeptide may bind to a receptor and cause the internalization and destruction of the receptor, thus preventing it from recycling to the membrane surface. Also, by binding to one receptor a chimeric polypeptide may cause another receptor or membrane protein to become desensitized or unable to carry out its normal function.

Chimeric chemokine polypeptides can also be used to prevent infection of cells by HIV or other viruses by blocking the binding of virus to chemokine receptors. The chimeric chemokine polypeptide including SDF-1 $\alpha$  and Fc polypeptides has been shown to bind to cells expressing the fusin/CXCR4 receptor. This binding will block HIV-1 isolates that are T-tropic from infecting fusin-positive cells in multiple ways: competing with HIV for existing chemokine receptors, down-regulation of the chemokine receptors by internalization, as well as desensitization of receptors required by HIV for infection. In a similar manner the construct consisting of an MIP-1 $\alpha$  or MIP-1 $\beta$  polypeptide and an Fc polypeptide will bind to cells expressing the CCR5 receptor. This binding will block HIV-1



isolates that are M-tropic from infecting CCR5-positive cells in multiple ways: competing with HIV for existing chemokine receptors, down-regulation of the chemokine receptors by internalization, as well as desensitization of receptors required by HIV for infection. Alterations of the chimeric polypeptide, such as additions of amino acids at the N-terminus of the chemokine domain, may result in enhanced binding with loss of signaling, resulting in strong antagonism. By making chimeric chemokine polypeptides with several different chemokines a wide range of chemokine receptors can be inhibited or desensitized, thus blocking viral isolates that have mutated to infect cells using other chemokine receptors. It is also possible to modify a chemokine sequence so that it will bind to a wider array of receptors; thus, one construct could bind to CCR5 as well as other CCR receptors and another construct could bind to CXCR4 as well as a variety of other CXCR receptors. By simultaneously administering a combination of chimeric chemokine polypeptides, the greatest number of chemokine receptor types could be protected from binding by HIV or other viral isolates.

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#### Administration and Dosing

A chimeric polypeptide of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to polypeptide and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, chemokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the polypeptide or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a

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synergistic effect with protein of the invention, or to minimize side effects. Conversely, polypeptides of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other  
5 hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A polypeptide of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a polypeptide of the invention in such multimeric or complexed form.

10 The pharmaceutical composition of the invention may be in the form of a complex of the polypeptide(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR)  
15 following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin  
20 and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other  
25 pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the  
30 art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728;

U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of polypeptide of the present invention is administered to an organism, preferably a mammal, having a condition to be treated. Chimeric polypeptides of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, polypeptides of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of polypeptides of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of polypeptide of the present invention is administered orally, polypeptide of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or

an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% polypeptide of the present invention, and preferably from about 25 to 90% polypeptide of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of polypeptide of the present invention, and preferably from about 1 to 50% polypeptide of the present invention.

When a therapeutically effective amount of polypeptide of the present invention is administered by intravenous, cutaneous or subcutaneous injection, polypeptide of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable polypeptide solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to chimeric polypeptide of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of polypeptide of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of chimeric polypeptide of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of polypeptide of the present invention and observe the patient's response. Larger doses of polypeptide of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should

contain about 0.01 ng to about 100 mg (preferably about 0.1  $\mu$ g to about 10 mg, more preferably about 0.1  $\mu$ g to about 1 mg) of polypeptide of the present invention per kg body weight.

5 The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the polypeptide of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the  
10 pharmaceutical composition of the present invention.

Polypeptide of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the chimeric polypeptide. Such antibodies may be obtained using either the entire chimeric polypeptide or fragments thereof as an immunogen, the fragments preferably comprising portions of  
15 both the chemokine and heterologous polypeptide domains. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal  
20 antibodies binding to the polypeptide of the invention may be useful diagnostic agents for the immunodetection of the polypeptide. Neutralizing monoclonal antibodies binding to the chimeric polypeptide may also be useful therapeutics for both conditions associated with the chemokine corresponding to the chemokine domain of the chimeric polypeptide and also in the treatment of some forms of cancer where abnormal expression of that chemokine  
25 is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the chimeric polypeptide may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the chemokine corresponding to the chemokine domain of the chimeric polypeptide.

For compositions of the present invention which are useful for bone, cartilage,  
30 tendon, or ligament regeneration, the therapeutic method includes administering the composition topically, systemically, or locally as an implant or device. When

administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage, or tissue damage. Topical administration may be suitable for wound healing and tissue repair.

5 Therapeutically useful agents other than a polypeptide of the invention which may also optionally be included in the composition as described above, may alternatively or additionally be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the polypeptide-containing composition to the  
10 site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance, and interface properties. The particular  
15 application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid, and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix  
20 components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to  
25 alter pore size, particle size, particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the chimeric polypeptide compositions from  
30 disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorption of the chimeric polypeptide from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the polypeptide the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, polypeptides of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- $\alpha$  and TGF- $\beta$ ), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with polypeptides of the present invention.

The dosage regimen of a polypeptide-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the chimeric polypeptides, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration, and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other polypeptides in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone

growth and/or repair, for example, X-rays, histomorphometric determinations, and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other  
5 known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of chimeric polypeptides of the present invention in order to proliferate or to produce a desired effect on or activity in such  
10 cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.

The following examples illustrate embodiments of the present invention, but are not intended to limit the scope of the disclosure.

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#### EXAMPLE 1 - CONSTRUCTION OF PLASMIDS ENCODING CHIMERIC POLYPEPTIDES

Plasmids containing chimeric gene constructions were created by ligating together four DNA fragments: a chemokine-encoding fragment, a fragment containing a linker and  
20 part of the Fc portion of the IgG4 gene, a fragment containing the rest of the Fc portion of the IgG4 gene, and a vector fragment. The resulting plasmid includes a chemokine-encoding sequence joined in-frame to a [Glycine-Serine]<sub>3</sub> linker sequence that is joined in-frame to the first codon for the hinge region of human IgG4. The Fc region of this chimeric gene is comprised of the hinge, CH2, and CH3 regions of human IgG4, including introns,  
25 and several bases downstream from the IgG4 stop codon. The Fc portion of this chimeric gene construct also includes two amino acid changes which result in reduced Fc-receptor binding and complement fixation.

#### Chemokine-encoding fragments:

30 The SDF1- $\alpha$  fragment of clones S1-2 and S1-3 was generated using PCR with human SDF-1 $\alpha$  cDNA as template, and with PCR primers adding NotI and BamHI sites to



the upstream and downstream ends of the SDF-1 $\alpha$  sequence, respectively. The SDF1- $\alpha$  fragment of clones S1-2 and S1-3 consists of eleven bases upstream of the initiating ATG of the signal sequence, through the final codon of the mature protein sequence. The DNA sequences of clones S1-2 and S1-3 appear to be identical. The SDF1- $\alpha$  fragment of clone  
5 SK2-2 was constructed similarly to that of S1-2 and S1-3, except that the upstream primer adding the NotI site extended through the signal sequence and into the mature protein coding sequence, with a deletion of the six nucleotides for amino acids 20 and 21 of the protein encoded by clones S1-2 and S1-3. The MIP1- $\alpha$  fragment of clones MP-1, MP-2, and MP-6 was generated using PCR with human MIP-1 $\alpha$  cDNA (the HUMCYTNEWA  
10 allele) as template, and with PCR primers adding NotI and BamHI sites to the upstream and downstream ends of the MIP-1 $\alpha$  sequence, respectively. The protein sequence for MIP-1 $\alpha$  sequence is that derived from HUMCYTNEWA (SEQ ID NO:9); there is another MIP-1 $\alpha$  allele which is not present in all humans, but HUMCYTNEWA is present in all humans. Some nucleotides were changed by the 3' MIP-1 $\alpha$  primer in the PCR, but these nucleotide  
15 changes do not alter the amino acid sequence. The DNA sequence of MP-1 has been determined and while the DNA sequences of MP-2 and MP-6 are anticipated to be identical to that of MP-1, these clones may contain some PCR-generated DNA sequence alterations. The MIP-1 $\alpha$  fragment of clones MP-1, MP-2, and MP-6 consists of fourteen bases upstream of the initiating ATG of the signal sequence, through the final codon of the mature protein  
20 sequence.

The MIP1- $\beta$  fragment of clone MPB-X is generated using PCR with human PHA-stimulated T-cell cDNA as template, and with PCR primers adding NotI and BamHI sites to the upstream and downstream ends of the MIP-1 $\beta$  sequence, respectively. The nucleotide and protein sequences for MIP-1 $\beta$  sequence are derived from HUMACT2A  
25 (SEQ ID NO:10). The MIP-1 $\beta$  fragment is predicted to consist of fifteen bases upstream of the initiating ATG of the signal sequence, through the final codon of the mature protein sequence.

#### Linker and partial IgG4 Fc fragment:

30 All of the chimeric gene constructions use the same DNA fragment encoding a [Glycine-Serine]<sub>3</sub> linker sequence and a portion of the Fc region of human IgG4.

Mutations were introduced into the IgG4 sequence, so that two amino acids in the CH2 region were changed from wild-type (in SEQ ID NO:1, 116 L has been changed to E, and 118 G has been changed to A, with corresponding nucleotide changes). The IgG4 sequence in this fragment contains an intron (nucleotides 346 to 463 in SEQ ID NO:2). The linker/partial Fc fragment was generated using PCR from the mutated human IgG4 sequence, with plasmid G081 (phhcd28.2higg4mcys) as template, and with one PCR primer adding a BamHI site and the Gly-Ser linker region to the 5' end and the other PCR primer adding a SacII site to the 3' end.

10 Remainder of IgG4 Fc fragment:

This DNA fragment was generated by restriction enzyme digestion with SacII and EcoRI from the plasmid G022 encoding human IgG4 (CD28-IgG4), and purified. The IgG4 sequence in this fragment contains an intron (nucleotides 794 to 890 in SEQ ID NO:2). In the human IgG4 sequence of this fragment, a base-pair change from wild-type IgG4 sequence (in SEQ ID NO:2, base 832, C has been changed to T) was found in the intron (non-coding) region, which is expected to have no effect on expression or composition of the gene product encoded by the chimeric gene construct.

Vector fragment:

20 This fragment was derived from the pED.Fc vector by digestion with NotI and EcoRI to remove the human IgG1 insert, resulting in a vector fragment with COS and CHO mammalian expression sequences that is similar to the pED vector.

EXAMPLE 2 - EXPRESSION AND PURIFICATION OF CHIMERIC POLYPEPTIDES

25 Chimeric polypeptides S1-3, SK2-2, MP1, MP2, and MP6, encoded by chimeric plasmid constructs, were expressed by transient expression in COS cells and released into the cell culture medium. COS 1 (clone M6) cells were transiently transfected with the appropriate plasmid, using Lipofectamine™ Reagent (GibcoBRL) and following the procedure given in the product insert, with the following modifications. COS cells are seeded into 100-mm tissue culture dishes 16-24 hours prior to transfection, at about 1-1.5 x 10<sup>6</sup> cells per plate, in complete DME medium (DME plus 10% fetal bovine serum, 2mM

glutamine, and 100 units each penicillin and streptomycin). All incubations of COS cells were at 37 degrees C in 10% CO<sub>2</sub>. For each culture dish of cells, 8 µg plasmid DNA and 48 µl Lipofectamine™ Reagent are mixed in 0.8 ml DME. After 30 minutes at room temperature, 3.2 ml DME (plus 2mM glutamine and 100 units each penicillin and streptomycin) are added to the DNA-Lipofectamine™ Reagent mixture, mixed, and layered on top of the DME-washed COS cells. After 18 to 24 hours of incubation, this medium is replaced with complete DME medium. After an additional 2 to 4 hours incubation, the COS plates are washed twice with 5-10 ml DME, and 10 ml DME medium without serum (plus 2mM glutamine) is added. After an additional 36 to 48 hours incubation the medium is collected, with any COS cells removed by centrifugation. The chimeric polypeptide MPB-X can also be expressed in a similar fashion.

The secreted chimeric polypeptide can be purified from this medium, or the medium can be used in various assays after quantitation of the amount of chimeric polypeptide by ELISA, using human IgG4 Kappa of known concentration to generate a standard curve.

Concentrations of the expressed chimeric polypeptides secreted into the cell culture medium were determined by ELISA using human IgG4 as a standard, and the results are shown in the table below.

Table 1

Plasmid Construct:	Concentration (µg/ml):	
	Experiment 1:	Experiment 2:
S1-2	0.8	not done
S1-3	1.5	5.0
SK2-2	1.0	5.5

Chimeric polypeptides were purified from cell culture supernatants by immunoprecipitation using Protein A Sepharose® (Pharmacia CL-4B). For example, chimeric polypeptides can be purified from 75 ml of conditioned medium by the following method. Adjust the conditioned medium to 50 mM Tris, pH 7.5. Add 100 mg Protein A Sepharose suspended in about 1 ml PBS. Incubate with rotation at 4 degrees C overnight.

Sodium azide may be added. Collect Protein A Sepharose by centrifugation, and transfer it to a BioRad Poly-Prep® column. Wash the Sepharose with 10 to 20 ml PBS. Elute the chimeric polypeptide with 12 mM HCl, and immediately neutralize the eluant by adjusting it to 50 mM Tris, pH 7.5. Elute in steps by suspending the Sepharose in 2 ml 12mM HCl and collecting 1 ml fractions. The amount of chimeric polypeptide in the fractions can be quantitated by ELISA.

Figure 1, panels A-D, depicts SDS-PAGE gels stained with Coomassie Blue demonstrating the expression of chimeric chemokine polypeptides in mammalian COS cells. The chimeric polypeptides were purified using protein-A, then electrophoresed on SDS-PAGE gels under reducing and non-reducing conditions. The SDF1- $\alpha$ -Fc chimeric polypeptides S1-3 and SK2-2 and the MIP-1 $\alpha$ -Fc chimeric polypeptides MP-1, MP-2, and MP-6 migrated as bands with a  $M_r$  of ~40 kD under reducing conditions and ~80 kD under non-reducing conditions.

### 15 EXAMPLE 3 - BINDING OF CHIMERIC POLYPEPTIDES TO CELLS EXPRESSING RECEPTORS

Several human cell lines have been stained using the SDF-Fc chimeric polypeptides, demonstrating binding of the chimeric polypeptides to receptors expressed by these cells. A typical binding assay is described below. Cells were incubated on ice for a short period of time (15-60 minutes) in media containing of 2-10% FCS, 0-0.02% BSA, 0-0.02% rabbit serum, and 0.02-0.1% azide. The SDF-Fc chimeric polypeptide was added at concentrations of 0.5-2  $\mu$ g/ml. After incubation with occasional mixing samples were washed with 5-6 mls of the above media. In parallel cells were stained with a mouse monoclonal antibody (12G5, IgG2a) specific for fusin/CXCR4, added at 5-20  $\mu$ g/ml. For negative controls a human IgG4 or a mouse IgG2a were used at 5-20  $\mu$ g/ml. The cells were then incubated for a short period of time with 100  $\mu$ l of a 1:100 dilution of the second or detecting antibody. The detecting antibody used was a goat anti-human IgG F(ab')<sub>2</sub> antibody (for the human IgG4 controls and the SDF-Fc samples) or a goat anti-mouse IgG F(ab')<sub>2</sub> antibody (for the mouse Ig controls, murine anti-human fusin, and murine anti-human cell-surface proteins or CD3) that was labeled with PE fluorescence. After another 15-60 minutes on ice with occasional mixing followed by an extensive wash with 5-6 ml

of staining media, the cells were resuspended in 400  $\mu$ l and analyzed using a FACSCAN (BD) fluorescence-activated cell analyzer.

Table 2 shows the results of staining Jurkat and U937 cells by binding anti-fusin antibody or the chimeric SDF-1 $\alpha$  chemokine polypeptides S1-3 or SK2-2 to them.

5 Detection of fusin/CXCR4 expression by a human T cell line and a human monocyte line using a fusin-specific mAb (12G5) is comparable to detection with SDF-Fc constructs SK2-2 or S1-3. Jurkat cells, derived from a patient with acute T cell leukemia, and U937, a macrophage-like cell line derived from a patient with histiocytic lymphoma, were used. About  $5 \times 10^5$  cells were added to 12x75 mm plastic tubes in 50  $\mu$ l of staining buffer

10 consisting of RPMI-1640 (phenol red free with 10 mM HEPES) or PBS containing 2% FCS, 2% rabbit serum, and 0.1% azide. Anti-fusin staining controls consisted of either media only or a mouse IgG2a control antibody and were equivalent in staining. The anti-fusin mAb 12G5 was added at a final concentration of 16  $\mu$ g/ml (Exp. 1) or 20  $\mu$ g/ml (Exp. 2) diluted in staining buffer. After 30 minutes on ice with mixing the cells were

15 washed with 5 ml of staining buffer and 100  $\mu$ l of a 1:100 dilution of Goat anti-mouse Ig PE (Southern Biotech) was added to detect cell-bound mouse antibody. For SDF-Fc staining the control consisted of either media only or human IgG4 antibody. The SDF-Fc constructs were added at a final concentration of 0.5  $\mu$ g/ml for the SK2-2 in Exp. 1 or at 1  $\mu$ g/ml for the S1-3 in Exp. 2. After 30 minutes on ice with mixing the cells were washed

20 with 5 ml of staining buffer and 100  $\mu$ l of a 1:100 dilution of goat anti-human Ig PE was added to detect cell-bound SDF-Fc. The control antibodies gave no increase over the second antibody only. The staining with anti-fusin (12G5) was equivalent to that seen with the SDF-Fc constructs, indicating that all human cells that were expressing the fusin receptor, as shown by anti-fusin antibody binding, also bound the SDF-Fc chimeric

25 polypeptides. Human RPMI 8866 cells that do not express fusin (as indicated by absence of staining) did not bind SDF-Fc chimeric polypeptides (data not shown).

The data shown for Exp. 1 in Table 2 corresponds to the results shown graphically as histograms in Figure 2. The x axis of the histograms (see Figure 2A) was divided into three regions: M1= channel 1-11; M2 = channel 11-123; and M3 = channel 123-1370. The

30 data is expressed as the percentage of appropriately gated cells in each of these regions. Also given in Table 2 is the peak channel and the median channel. The peak channel is the

channel containing the highest distribution of cells. The median is the channel where 50 % of the cells are to the right or left of this point. Figure 2, panels A-D represent the histograms for Exp. 1 comparing the anti-fusin antibody 12G5 to the chimeric polypeptide SK2-2. The thinner line is that for the control while the thicker line is that for the 12G5 (Fig 2A and 2C) or for SK2-2 (Fig 2B and 2D). Figure 2A and 2B indicate staining of Jurkat cells. Figure 2C and 2D indicate staining of U937 cells.

Table 2. Jurkat and U937 cell staining by anti-Fusin mAb and SDF-Fc constructs

% of Cells in Each Channel Range					
	M1	M2	M3	Peak Channel	Median Channel
Channel Range:	1-11	11-123	123-1370		
Exp. 1					
Jurkat Control	97.5%	2.5%	0 %	3	3
Jurkat anti-Fusin	0.5%	21.4%	78.2 %	281	225
Jurkat Control	98.1 %	1.9 %	0 %	3	3
Jurkat SK2-2	2.4 %	42.3 %	56.1 %	145	132
U937 Control	97.8 %	2.2 %	0 %	3	3
U937 anti-Fusin	0.3%	71.8 %	28.4 %	81	95
U937 Control	99.6 %	0.5 %	0 %	3	3
U937 SK2-2	2.3 %	72.6 %	25.5 %	47	63
Exp. 2					
Jurkat Control	91.6%	8.5%	0.1 %	5	5
Jurkat anti-Fusin	0.4%	48.1%	51.9 %	121	134
Jurkat Control	86.3 %	13.9 %	0 %	6	6
Jurkat S1-3	6.5%	51.3%	42.8%	139	106
U937 Control	98.9%	1.1%	0 %	4	4
U937 anti-Fusin	0.2%	53%	47.5%	114	118
U937 Control	98%	2.0%	0 %	5	5
U937 S1-3	1.0%	77.3%	22.1 %	62	78

Table 3. Lymphocyte and dendritic cell staining by anti-Fusin mAb and SDF-Fc constructs

% of Cells in Each Channel Range						
	M1	M2	M3	Peak Channel	Median Channel	
Channel Range:	1-11	11-123	123-1370			
5	T lymphocytes					
	Control *	74.8 %	2.3 %	0 %	1	2
	anti-CD3	2.8 %	4.2 %	88.7 %	610	523
	anti-Fusin	39.3 %	56.9 %	3.2 %	1	18
	IgG4 Control **	79.8 %	1.8 %	0.2 %	1	2
10	SDF-Fc SK2-2	27.2 %	70.5 %	2.5 %	37	21
	SDF-Fc S1-2	31.5 %	66.4 %	2.3 %	31	20
Dendritic Cells and other Adherent Cells						
	Control *	38.6 %	60.5 %	1.5 %	13	13
	anti-Fusin	16.1 %	59.0 %	25.1 %	12	31
15	IgG4 Control	53.1 %	47.3 %	0.1 %	8	11
	SDF-Fc SK2-2	18.6 %	77.6 %	4.3 %	10	23

\* Mouse gamma 2a control + Goat anti-Mouse PE second step

\*\* Human IgG4 control + Goat anti-Human PE second step

20 Table 3 shows the results of staining T lymphocytes isolated from peripheral blood, and dendritic and other adherent cells isolated from human bone marrow (following culture in media containing IL-4 and GMSF then TNF), by binding anti-fusin antibody or the chimeric SDF-1 $\alpha$  chemokine polypeptides S1-3 or SK2-2 to them. These results indicate that a variety of cells expressing the fusin receptor bind the chimeric SDF-1 $\alpha$

25 chemokine polypeptides.

Table 4. Effect of adding Human SDF-1 $\beta$  during staining of U937 cells  
by Anti-Fusin mAb and chimeric SDF-Fc constructs

		% of Cells in Each Channel				
		Range				
		M1	M2	M3	Peak Channel	Median Channel
5	Channel Range:	1-11	11-123	123- 1370		
10	Control *	93.5 %	5.7 %	0.8 %	3	3
	anti-Fusin	0 %	41.3 %	58.8 %	157	136
	50 ng/ml Human SDF-1 $\beta$ + anti-Fusin	0 %	47.1 %	52.9 %	106	127
	500 ng/ml Human SDF-1 $\beta$ + anti-Fusin	0 %	70.0 %	30.0 %	95	95
	IgG4 Control **	99.0 %	1.0 %	0 %	3	3
	SDF-Fc SK2-2	2.0 %	97.0 %	1.5 %	47	47
	50 ng/ml Human SDF-1 $\beta$ + SDF-Fc SK2-2	1.6 %	97 %	1.6 %	45	46
	500 ng/ml Human SDF-1 $\beta$ + SDF-Fc SK2-2	8.4%	91.1 %	0.7 %	29	30
	SDF-Fc S1-3	1.4 %	96.7 %	1.9 %	48	47
	50 ng/ml Human SDF-1 $\beta$ + SDF-Fc S1-2	2.0 %	96.7 %	1.9 %	42	46
15	500 ng/ml Human SDF-1 $\beta$ + SDF-Fc S1-2	11.0 %	88.7 %	0.3 %	30	26

\* Mouse gamma 2a control + Goat anti-Mouse PE second step

\*\* Human IgG4 control + Goat anti-Human PE second step

20 For the experiment shown in Table 4, purified human SDF-1 $\beta$  chemokine, prepared in *E. coli* and containing an N-terminal methionine residue, was mixed with either anti-fusin antibody or chimeric SDF-1 $\alpha$  polypeptide, then incubated with cells on ice in the presence of azide. The results shown in Table 4 indicate that a 10-fold increase in the amount of SDF-1 $\beta$  chemokine eliminates some anti-fusin antibody binding to cells, but  
25 does not reduce the amount of chimeric SDF-Fc polypeptide binding to cells. This suggests that the affinity of the chimeric SDF-Fc polypeptide for its binding site on cells, presumably the fusin receptor, is sufficiently high that it cannot be competed off by addition of excess SDF-1 $\beta$  chemokine.

30 Binding of the MIP-1 $\alpha$ -Fc and MIP-1 $\beta$ -Fc chimeric polypeptides to cells is determined by a cell-staining assay analogous to that described above.



#### EXAMPLE 4 - ALTERATION OR INHIBITION OF CALCIUM FLUX BY CHIMERIC POLYPEPTIDES

When chemokines bind to receptors present within the membranes of cells, a calcium flux may be induced. When chimeric chemokine polypeptides bind to these  
5 receptors, the duration, intensity, or other properties of the calcium flux may be altered, or the calcium flux may be inhibited. This calcium flux may be measured using the following protocol, and the effects of chemokine and chimeric chemokine polypeptide binding to receptors compared.

Harvest the cells, wash twice in first wash buffer (10 mM MOPS or HEPES at  
10 about pH7.2, 1 mM  $\text{CaCl}_2$ , 1 mM glucose, 140 mM NaCl), adjust to  $10^7$  cells per ml, resuspend in loading/FACS buffer (10 mM MOPS or HEPES at about pH7.2, 1 mM  $\text{CaCl}_2$ , 1 mM glucose, 140 mM NaCl, 0.2% BSA). Dissolve 50  $\mu\text{g}$  vial of FLUO-3 ester (Molecular Probes, cat. #F-1242) in 50  $\mu\text{l}$  DMSO right before use. Add 5  $\mu\text{l}$  FLUO-3 ester (approximately 5  $\mu\text{M}$ , different concentrations may be needed for different cell types) for  
15 each ml of cells. Incubate for 20-30 minutes at room temperature. Wash twice in medium (for example, RPMI with fetal calf serum). Resuspend cells at  $10^7$  per ml in medium (or loading/FACS buffer). Store on ice until ready to use (or store at room temperature). To test for calcium flux, dilute cells into loading/FACS buffer, 100  $\mu\text{l}$  of cells per 1 ml buffer. Using a FACSCAN (BD) fluorescence-activated cell analyzer, determine the background  
20 reading for the loaded cells (use FL1 channel; set maximum signal at about 200). Stimulate appropriately (with one or more reagents, sequentially) and read on FACS for 3-15 minutes or more, watching for an increase in fluorescence due to calcium flux. The ionophore ionomycin can be used as a positive control to demonstrate that the cells being tested are capable of demonstrating a calcium flux.

25

#### EXAMPLE 5 - STIMULATION OF CHEMOTAXIS BY CHIMERIC POLYPEPTIDES

Chimeric chemokine polypeptides can be tested for their ability to stimulate chemotaxis by any of the following assays for chemotactic activity. These assays (which  
will identify proteins that induce or prevent chemotaxis) measure the ability of a protein to  
30 induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for

movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed. by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. by Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28); Taub et al., J. Clin. Invest. 5 95:1370-1376, 1995; Lind et al., APMIS 103:140-146, 1995; Muller et al., Eur. J. Immunol. 25: 1744-1748; Gruber et al., J. of Immunol. 152:5860-5867, 1994; Johnston et al., J. of Immunol. 153: 1762-1768, 1994; all of which are incorporated herein by reference.

10 EXAMPLE 6 - DOWN-MODULATION OF RECEPTOR BY CHIMERIC POLYPEPTIDE  
BINDING

The ability of the chimeric SDF-Fc polypeptides to down-modulate chemokine receptors was compared with that of human SDF-1 $\beta$ . Jurkat cells were incubated for 3 hours or 15 hours at 37 degrees C with either human SDF-1 $\beta$  or chimeric SDF-Fc polypeptide, followed by a wash of the cells and staining with anti-fusin antibody as 15 described in Example 3. Mock experiments involved incubating cells with COS cell supernatant containing neither SDF-1 $\beta$  nor chimeric SDF-Fc polypeptide. The results of these experiments are shown in Table 5.

Table 5. Down-Modulation of Fusin/CXCR4 by Incubation with Human SDF-1 $\beta$  or chimeric SDF-FC

% of Cells in Each Channel Range						
	M1	M2	M3	Peak Channel	Median Channel	
Channel Range:	1-11	11-123	123-1370			
5	Jurkat 3-hour Incubation					
	Media control	97.5%	2.5%	0 %	3	3
	Anti-fusin Media	0.4%	20.2%	79.6%	281	231
	500ng/ml Human SDF1 $\beta$	14.1%	83.2%	3.1%	20	22.3
	Mock	0.4%	12.3%	87.2%	276	302
10	70ng/ml SDF-Fc S1-2	6.1%	91.7%	2.5%	23	26
	140ng/ml SDF-Fc S1-3	15.5%	82.5%	2.4%	13	20
	90ng/ml SDF-Fc SK2-2	11.3%	86.8%	2.4%	23	22
	Jurkat 15-hour Incubation					
	Media control	97.5%	2.5%	0 %	3	3
	Anti-fusin Media	0.4%	20.2%	79.6%	281	231
	500ng/ml Human SDF1 $\beta$	1.7%	77.7%	20.9%	66	75
	70ng/ml SDF-FC S-2	5.9%	91.7%	2.7%	35	34
	140ng/ml SDF-FC S1-3	9.2%	90.0%	1.1%	26	25
15	90ng/ml SDF-FC SK2-2	7.0%	91.7%	1.5%	38	28

The apparent down-modulation of fusin receptor by human SDF-1 $\beta$  is not simply due to blocking of staining by the anti-fusin antibody by the binding of SDF-1 $\beta$  to fusin, since the results shown in Table 4 above indicate that the presence of human SDF-1 $\beta$  does not prevent anti-fusin binding to the extent observed here. The down-modulation by the chimeric SDF-Fc polypeptide is demonstrated by the failure of anti-fusin antibody to bind after incubation with this chimeric polypeptide (Table 5) and the weak staining of these cells with PE-labeled goat anti-human Ig to detect chimeric SDF-Fc polypeptide remaining after the 3- or 15-hour incubation (data not shown).

Down-regulation of receptor by binding of MIP-1 $\alpha$ -Fc and MIP-1 $\beta$ -Fc chimeric polypeptides to cells is determined by an assay for receptor down-regulation analogous to that described above.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

(i) APPLICANT: Herrmann, Steve  
Swanberg, Stephen

10

(ii) TITLE OF INVENTION: CHIMERIC POLYPEPTIDES CONTAINING  
CHEMOKINE DOMAINS

(iii) NUMBER OF SEQUENCES: 10

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

30

(viii) ATTORNEY/AGENT INFORMATION:

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(C) REFERENCE/DOCKET NUMBER: GI5291

35

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40

## (2) INFORMATION FOR SEQ ID NO:1:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 328 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5	Met Asn Ala Lys Val Val Val Val Leu Val Leu Val Leu Thr Ala Leu	1 5 10 15
	Cys Leu Ser Asp Gly Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys	20 25 30
10	Arg Phe Phe Glu Ser His Val Ala Arg Ala Asn Val Lys His Leu Lys	35 40 45
	Ile Leu Asn Thr Pro Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys	50 55 60
15	Asn Asn Asn Arg Gln Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln	65 70 75 80
	Glu Tyr Leu Glu Lys Ala Leu Asn Lys Gly Ser Gly Ser Gly Ser Gly	85 90 95
20	Ser Gly Ser Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala	100 105 110
	Pro Glu Phe Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro	115 120 125
25	Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val	130 135 140
	Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val	145 150 155 160
	Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln	165 170 175
35	Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln	180 185 190
	Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly	195 200 205
40	Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro	210 215 220
	Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr	225 230 235 240
	Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser	245 250 255
50	Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr	260 265 270
	Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr	

275

280

285

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe  
290 295 300

5

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
305 310 315 320

Ser Leu Ser Leu Ser Leu Gly Lys  
325

10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 1222 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GCGGCCGCGC CATGAACGCC AAGGTCGTGG TCGTGCTGGT CCTCGTGCTG ACCGCGCTCT 60

GCCTCAGCGA CGGGAAGCCC GTCAGCCTGA GCTACAGATG CCCATGCCGA TTCTTCGAAA 120

30

GCCATGTTGC CAGAGCCAAC GTCAAGCATC TCAAAATTCT CAACACTCCA AACTGTGCCC 180

TTCAGATTGT AGCCCGGCTG AAGAACAACA ACAGACAAGT GTGCATTGAC CCGAAGCTAA 240

35

AGTGGATTCA GGAGTACCTG GAGAAAGCTT TAAACAAGGG ATCCGGCTCT GGGAGCGGCT 300

CTGGCTCTGA GTCCAAATAT GGTCCCCCAT GCCCATCATG TCCAGGTAAG CCAACCCAGG 360

CCTCGCCCTC CAGCTCAAGG CGGGACAGGT GCCCTAGAGT AGCCTGCATC CAGGGACAGG 420

40

CCCCAGCCGG GTGCTGACGC ATCCACCTCC ATCTCTTCCT CAGCACCTGA GTTCGAGGGG 480

GCACCATCAG TCTTCCTGTT CCCCCAAAA CCAAGGACA CTCTCATGAT CTCCCGGACC 540

45

CCTGAGGTCA CGTGCGTGGT GGTGGACGTG AGCCAGGAAG ACCCCGAGGT CCAGTTCAAC 600

TGGTACGTGG ATGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTTC 660

AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCTGTC ACCAGGACTG GCTGAACGGC 720

50

AAGGAGTACA AGTGCAAGGT CTCCAACAAA GGCCTCCCGT CCTCCATCGA GAAAACCATC 780

TCCAAAGCCA AAGGTGGGAC CCACGGGGTG CGAGGGCCAC ACGGACAGAG GTCAGCTCGG 840

CCCACCCTCT GCCCTGGGAG TGACCGCTGT GCCAACCTCT GTCCCTACAG GGCAGCCCCG 900  
 AGAGCCACAG GTGTACACCC TGCCCCATC CCAGGAGGAG ATGACCAAGA ACCAGGTCAG 960  
 5 CCTGACCTGC CTGGTCAAAG GCTTCTACCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA 1020  
 TGGGCAGCCG GAGAACAACCT ACAAGACCAC GCCTCCCGTG CTGGAATCCG ACGGCTCCTT 1080  
 CTTCTCTTAC AGCAGGCTAA CCGTGGACAA GAGCAGGTGG CAGGAGGGGA ATGTCTTCTC 1140  
 10 ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACA CAGAAGAGCC TCTCCCTGTC 1200  
 TCTGGGTAAA TGATAAGAAT TC 1222

15 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 326 amino acids  
 (B) TYPE: amino acid  
 20 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30 Met Asn Ala Lys Val Val Val Val Leu Val Leu Val Leu Thr Ala Leu  
 1 5 10 15  
 Cys Leu Ser Lys Pro Val Ser Leu Ser Tyr Arg Cys Pro Cys Arg Phe  
 20 25 30  
 35 Phe Glu Ser His Val Ala Arg Ala Asn Val Lys His Leu Lys Ile Leu  
 35 40 45  
 40 Asn Thr Pro Asn Cys Ala Leu Gln Ile Val Ala Arg Leu Lys Asn Asn  
 50 55 60  
 Asn Arg Gln Val Cys Ile Asp Pro Lys Leu Lys Trp Ile Gln Glu Tyr  
 65 70 75 80  
 45 Leu Glu Lys Ala Leu Asn Lys Gly Ser Gly Ser Gly Ser Gly Ser Gly  
 85 90 95  
 Ser Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu  
 100 105 110  
 50 Phe Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 115 120 125  
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp

130 135 140

Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly  
 145 150 155 160

5 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn  
 165 170 175

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 180 185 190

10 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro  
 195 200 205

15 Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn  
 225 230 235 240

20 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
 260 265 270

25 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg  
 275 280 285

30 Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys  
 290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 305 310 315 320

35 Ser Leu Ser Leu Gly Lys  
 325

## (2) INFORMATION FOR SEQ ID NO:4:

40

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1216 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

45

## (ii) MOLECULE TYPE: cDNA

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGGCCGCGC CATGAACGCC AAGGTCGTGG TCGTGCTGGT CCTCGTGCTG ACCGCGCTCT

60



GCCTCAGCAA GCCCGTCAGC CTGAGCTACA GATGCCCATG CCGATTCTTC GAAAGCCATG 120  
 TTGCCAGAGC CAACGTCAAG CATCTCAAAA TTCTCAACAC TCCAAACTGT GCCCTTCAGA 180  
 5 TTGTAGCCCG GCTGAAGAAC AACAACAGAC AAGTGTGCAT TGACCCGAAG CTAAAGTGGA 240  
 TTCAGGAGTA CCTGGAGAAA GCTTTAAACA AGGGATCCGG CTCTGGGAGC GGCTCTGGCT 300  
 CTGAGTCCAA ATATGGTCCC CCATGCCCAT CATGTCCAGG TAAGCCAACC CAGGCCTCGC 360  
 10 CCTCCAGCTC AAGGCGGGAC AGGTGCCCTA GAGTAGCCTG CATCCAGGGA CAGGCCCCAG 420  
 CCGGGTGCTG ACGCATCCAC CTCCATCTCT TCCTCAGCAC CTGAGTTCGA GGGGGCACCA 480  
 15 TCAGTCTTCC TGTTCCCCC AAAACCCAAG GACACTCTCA TGATCTCCCG GACCCCTGAG 540  
 GTCACGTGCG TGGTGGTGA CGTGAGCCAG GAAGACCCG AGGTCCAGTT CAACTGGTAC 600  
 GTGGATGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCGC GGGAGGAGCA GTTCAACAGC 660  
 20 ACGTACCGTG TGGTCAGCGT CCTCACCCTG CTGCACCAGG ACTGGCTGAA CGGCAAGGAG 720  
 TACAAGTGCA AGGTCTCCAA CAAAGGCCTC CCGTCCTCCA TCGAGAAAAC CATCTCCAAA 780  
 25 GCCAAAGGTG GGACCCACGG GGTGCGAGGG CCACACGGAC AGAGGTCAGC TCGGCCACC 840  
 CTCTGCCCTG GGAGTGACCG CTGTGCCAAC CTCTGTCCCT ACAGGGCAGC CCCGAGAGCC 900  
 ACAGGTGTAC ACCCTGCCCC CATCCAGGA GGAGATGACC AAGAACCAGG TCAGCCTGAC 960  
 30 CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA 1020  
 GCCGGAGAAC AACTACAAGA CCACGCCTCC CGTGCTGGAC TCCGACGGCT CCTTCTTCCT 1080  
 35 CTACAGCAGG CTAACCGTGG ACAAGAGCAG GTGGCAGGAG GGAATGTCT TCTCATGCTC 1140  
 CGTGATGCAT GAGGCTCTGC ACAACCACTA CACACAGAAG AGCCTCTCCC TGTCTCTGGG 1200  
 TAAATGATAA GAATTC 1216

40

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

45

- (A) LENGTH: 331 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

	Met	Gln	Val	Ser	Thr	Ala	Ala	Leu	Ala	Val	Leu	Leu	Cys	Thr	Met	Ala	
	1				5					10					15		
5	Leu	Cys	Asn	Gln	Phe	Ser	Ala	Ser	Leu	Ala	Ala	Asp	Thr	Pro	Thr	Ala	
				20					25					30			
	Cys	Cys	Phe	Ser	Tyr	Thr	Ser	Arg	Gln	Ile	Pro	Gln	Asn	Phe	Ile	Ala	
			35					40					45				
10	Asp	Tyr	Phe	Glu	Thr	Ser	Ser	Gln	Cys	Ser	Lys	Pro	Gly	Val	Ile	Phe	
	50						55					60					
	Leu	Thr	Lys	Arg	Ser	Arg	Gln	Val	Cys	Ala	Asp	Pro	Ser	Glu	Glu	Trp	
	65					70					75					80	
15	Val	Gln	Lys	Tyr	Val	Ser	Asp	Leu	Glu	Leu	Ser	Ala	Gly	Ser	Gly	Ser	
					85				90						95		
	Gly	Ser	Gly	Ser	Gly	Ser	Glu	Ser	Lys	Tyr	Gly	Pro	Pro	Cys	Pro	Ser	
20				100					105					110			
	Cys	Pro	Ala	Pro	Glu	Phe	Glu	Gly	Ala	Pro	Ser	Val	Phe	Leu	Phe	Pro	
			115					120					125				
25	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	
		130					135					140					
	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	
	145					150					155					160	
30	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	
					165					170					175		
	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	
35				180					185					190			
	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	
			195					200					205				
40	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	
		210					215					220					
	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Gln	Glu	
	225					230					235					240	
45	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	
				245					250						255		
	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	
50				260					265					270			
	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	
			275					280					285				

Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly  
 290 295 300

5 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr  
 305 310 315 320

Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
 325 330

10 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 1234 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

25 GCGGCCGCCC AATCATGCAG GTCTCCACTG CTGCCCTTGC TGTCTCCTC TGCACCATGG 60  
 CTCTCTGCAA CCAGTTCTCT GCATCACTTG CTGCTGACAC GCCGACCGCC TGCTGCTTCA 120  
 GCTACACCTC CCGGCAGATT CCACAGAATT TCATAGCTGA CTACTTTGAG ACGAGCAGCC 180  
 30 AGTGCTCCAA GCCCGGTGTC ATCTTCCTAA CCAAGCGAAG CCGGCAGGTC TGTGCTGACC 240  
 CCAGTGAGGA GTGGGTCCAG AAATACGTCA GTGACCTGGA GCTGAGTGCC GGATCCGGCT 300  
 35 CTGGGAGCGG CTCTGGCTCT GAGTCCAAAT ATGGTCCCCC ATGCCCATCA TGTCCAGGTA 360  
 AGCCAACCCA GGCCTCGCCC TCCAGCTCAA GGCGGGACAG GTGCCCTAGA GTAGCCTGCA 420  
 TCCAGGGACA GGCCCCAGCC GGGTGCTGAC GCATCCACCT CCATCTCTTC CTCAGCACCT 480  
 40 GAGTTCGAGG GGGCACCATC AGTCTTCCTG TCCCCCCAA AACCCAAGGA CACTCTCATG 540  
 ATCTCCCGGA CCCCTGAGGT CACGTGCGTG GTGGTGGACG TGAGCCAGGA AGACCCCGAG 600  
 45 GTCCAGTTCA ACTGGTACGT GGATGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG 660  
 GAGGAGCAGT TCAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCTT GCACCAGGAC 720  
 TGGCTGAACG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGGCCTCCC GTCCTCCATC 780  
 50 GAGAAAACCA TCTCCAAAGC CAAAGGTGGG ACCCACGGGG TGCGAGGGCC ACACGGACAG 840  
 AGGTCAGCTC GGCCACCCCT CTGCCCTGGG AGTGACCGCT GTGCCAACCT CTGTCCCTAC 900

AGGGCAGCCC CGAGAGCCAC AGGTGTACAC CCTGCCCCCA TCCCAGGAGG AGATGACCAA 960  
 GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAC CCCAGCGACA TCGCCGTGGA 1020  
 5 GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG TGCTGGACTC 1080  
 CGACGGCTCC TTCTTCCTCT ACAGCAGGCT AACCGTGAC AAGAGCAGGT GGCAGGAGGG 1140  
 GAATGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CACAGAAGAG 1200  
 10 CCTCTCCCTG TCTCTGGGTA AATGATAAGA ATTC 1234

## (2) INFORMATION FOR SEQ ID NO:7:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 331 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Lys Leu Cys Val Thr Val Leu Ser Leu Leu Met Leu Val Ala Ala  
 1 5 10 15  
 30 Phe Cys Ser Pro Ala Leu Ser Ala Pro Met Gly Ser Asp Pro Pro Thr  
 20 25 30  
 35 Ala Cys Cys Phe Ser Tyr Thr Ala Arg Lys Leu Pro Arg Asn Phe Val  
 35 40 45  
 Val Asp Tyr Tyr Glu Thr Ser Ser Leu Cys Ser Gln Pro Ala Val Val  
 50 55 60  
 40 Phe Gln Thr Lys Arg Ser Lys Gln Val Cys Ala Asp Pro Ser Glu Ser  
 65 70 75 80  
 Trp Val Gln Glu Tyr Val Tyr Asp Leu Glu Leu Asn Gly Ser Gly Ser  
 85 90 95  
 45 Gly Ser Gly Ser Gly Ser Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser  
 100 105 110  
 Cys Pro Ala Pro Glu Phe Glu Gly Ala Pro Ser Val Phe Leu Phe Pro  
 50 115 120 125  
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr  
 130 135 140

	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	
	145					150					155					160	
5	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	
					165					170					175		
	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	
					180					185					190		
10	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	
					195			200					205				
	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	
15		210					215					220					
	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Gln	Glu	
	225					230					235					240	
20	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	
					245					250					255		
	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	
					260				265					270			
25	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	
					275			280					285				
	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Glu	Gly	
30		290					295					300					
	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	
	305					310					315					320	
35	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly	Lys						
					325					330							

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- 40 (A) LENGTH: 1235 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: cDNA

## 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGGCCGCCA ATACCATGAA GCTCTGCGTG ACTGTCCTGT CTCTCCTCAT GCTAGTAGCT 60

GCCTTCTGCT CTCCAGCGCT CTCAGCACCA ATGGGCTCAG ACCCTCCCAC CGCCTGCTGC 120

TTTTCTTACA CCGCGAGGAA GCTTCCTCGC AACTTTGTGG TAGATTACTA TGAGACCAGC 180  
AGCCTCTGCT CCCAGCCAGC TGTGGTATTC CAAACCAAAA GAAGCAAGCA AGTCTGTGCT 240  
5 GATCCCAAGT AATCCTGGGT CCAGGAGTAC GTGTATGACC TGGAAGTAA CGGATCCGGC 300  
TCTGGGAGCG GCTCTGGCTC TGAGTCCAAA TATGGTCCCC CATGCCATC ATGTCCAGGT 360  
AAGCCAACCC AGGCCTCGCC CTCCAGCTCA AGGCGGGACA GGTGCCCTAG AGTAGCCTGC 420  
10 ATCCAGGGAC AGGCCCCAGC CGGGTGCTGA CGCATCCACC TCCATCTCTT CCTCAGCACC 480  
TGAGTTCGAG GGGGCACCAT CAGTCTTCCT GTTCCCCCA AAACCCAAGG ACACTCTCAT 540  
15 GATCTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCAGG AAGACCCCGA 600  
GGTCCAGTTC AACTGGTACG TGGATGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG 660  
GGAGGAGCAG TTCAACAGCA CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA 720  
20 CTGGCTGAAC GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CGTCCTCCAT 780  
CGAGAAAACC ATCTCCAAAG CCAAAGGTGG GACCCACGGG GTGCGAGGGC CACACGGACA 840  
25 GAGGTCAGCT CGGCCACCC TCTGCCCTGG GAGTGACCGC TGTGCCAACC TCTGTCCCTA 900  
CAGGGCAGCC CCGAGAGCCA CAGGTGTACA CCCTGCCCCC ATCCCAGGAG GAGATGACCA 960  
AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA CCCCAGCGAC ATCGCCGTGG 1020  
30 AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT 1080  
CCGACGGCTC CTTCTTCCTC TACAGCAGGC TAACCGTGA CAAGAGCAGG TGGCAGGAGG 1140  
35 GGAATGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC ACACAGAAGA 1200  
GCCTCTCCCT GTCTCTGGGT AAATGATAAG AATTC 1235

## (2) INFORMATION FOR SEQ ID NO:9:

40

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 92 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

45

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Gln Val Ser Thr Ala Ala Leu Ala Val Leu Leu Cys Thr Met Ala

	1		5		10		15									
	Leu	Cys	Asn	Gln	Phe	Ser	Ala	Ser	Leu	Ala	Ala	Asp	Thr	Pro	Thr	Ala
				20					25						30	
5																
	Cys	Cys	Phe	Ser	Tyr	Thr	Ser	Arg	Gln	Ile	Pro	Gln	Asn	Phe	Ile	Ala
			35					40					45			
10	Asp	Tyr	Phe	Glu	Thr	Ser	Ser	Gln	Cys	Ser	Lys	Pro	Gly	Val	Ile	Phe
		50					55					60				
	Leu	Thr	Lys	Arg	Ser	Arg	Gln	Val	Cys	Ala	Asp	Pro	Ser	Glu	Glu	Trp
	65					70					75					80
15	Val	Gln	Lys	Tyr	Val	Ser	Asp	Leu	Glu	Leu	Ser	Ala				
					85					90						

## (2) INFORMATION FOR SEQ ID NO:10:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 92 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	Met	Lys	Leu	Cys	Val	Thr	Val	Leu	Ser	Leu	Leu	Met	Leu	Val	Ala	Ala
	1				5					10					15	
35																
	Phe	Cys	Ser	Pro	Ala	Leu	Ser	Ala	Pro	Met	Gly	Ser	Asp	Pro	Pro	Thr
				20					25					30		
40	Ala	Cys	Cys	Phe	Ser	Tyr	Thr	Ala	Arg	Lys	Leu	Pro	Arg	Asn	Phe	Val
		35						40					45			
	Val	Asp	Tyr	Tyr	Glu	Thr	Ser	Ser	Leu	Cys	Ser	Gln	Pro	Ala	Val	Val
		50					55					60				
45																
	Phe	Gln	Thr	Lys	Arg	Ser	Lys	Gln	Val	Cys	Ala	Asp	Pro	Ser	Glu	Ser
	65					70					75					80
	Trp	Val	Gln	Glu	Tyr	Val	Tyr	Asp	Leu	Glu	Leu	Asn				
					85					90						
50																

## WHAT IS CLAIMED IS:

1. A composition comprising an isolated polynucleotide encoding a chimeric polypeptide, the chimeric polypeptide comprising at least one chemokine polypeptide covalently attached to at least one heterologous polypeptide.
2. The composition of claim 1 wherein the heterologous polypeptide is covalently attached to the amino terminus of the chemokine polypeptide.
3. The composition of claim 2 wherein the encoded chimeric polypeptide comprises a linker polypeptide covalently attached to the heterologous polypeptide and the chemokine polypeptide.
4. The composition of claim 1 wherein the heterologous polypeptide is covalently attached to the carboxyl terminus of the chemokine polypeptide.
5. The composition of claim 4 wherein the encoded chimeric polypeptide comprises a linker polypeptide covalently attached to the heterologous polypeptide and the chemokine polypeptide.
6. The composition of claim 1 wherein the chemokine polypeptide is derived from SDF-1 $\alpha$ .
7. The composition of claim 1 wherein the chemokine polypeptide is SDF-1 $\alpha$ .
8. The composition of claim 1 wherein the chemokine polypeptide is derived from MIP-1 $\alpha$ .
9. The composition of claim 1 wherein the chemokine polypeptide is MIP-1 $\alpha$ .



10. The composition of claim 1 wherein the chemokine polypeptide is derived from MIP-1 $\beta$ .
11. The composition of claim 1 wherein the chemokine polypeptide is MIP-1 $\beta$ .
12. The composition of claim 1 wherein the heterologous polypeptide is an Fc polypeptide.
13. The composition of claim 1 wherein the polynucleotide is selected from the group consisting of:
  - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 12 to nucleotide 1213;
  - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 69 to nucleotide 1213;
  - (c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 72 to nucleotide 1213;
  - (d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:2 from nucleotide 75 to nucleotide 1213;
  - (e) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:2;
  - (f) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone S1-3 deposited under accession number ATCC 98338;
  - (g) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone S1-3 deposited under accession number ATCC 98338;
  - (h) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:1;

- (i) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:1 from amino acid 20 to amino acid 328;
- (j) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:1 from amino acid 22 to amino acid 328;
- (k) a polynucleotide encoding a chimeric polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:1;
- (l) a polynucleotide comprising a nucleotide sequence complementary to any one of the polynucleotides specified in (a)-(k) above; and
- (m) a polynucleotide capable of simultaneously hybridizing under stringent conditions to sequences encoding the chemokine polypeptide and to sequences encoding the heterologous polypeptide in any one of the polynucleotides specified in (a)-(l) above.

14. The composition of claim 1 wherein the polynucleotide is selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:4 from nucleotide 12 to nucleotide 1207;
- (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:4 from nucleotide 69 to nucleotide 1207;
- (c) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:4;
- (d) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone SK2-2 deposited under accession number ATCC 98339;
- (e) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone SK2-2 deposited under accession number ATCC 98339;
- (f) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:3;

(g) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:3 from amino acid 20 to amino acid 326;

(h) a polynucleotide encoding a chimeric polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:3;

(i) a polynucleotide comprising a nucleotide sequence complementary to any one of the polynucleotides specified in (a)-(h) above; and

(j) a polynucleotide capable of simultaneously hybridizing under stringent conditions to sequences encoding the chemokine polypeptide and to sequences encoding the heterologous polypeptide in any one of the polynucleotides specified in (a)-(i) above.

15. The composition of claim 1 wherein the polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:6 from nucleotide 15 to nucleotide 1225;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:6 from nucleotide 81 to nucleotide 1225;

(c) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:6;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone MP-1 deposited under accession number ATCC 98341;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone MP-2 deposited under accession number ATCC 98342;

(f) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone MP-6 deposited under accession number ATCC 98340;

(g) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone MP-1 deposited under accession number ATCC 98341;

(h) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone MP-2 deposited under accession number ATCC 98342;

(i) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone MP-6 deposited under accession number ATCC 98340;

(j) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:5;

(k) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:5 from amino acid 23 to amino acid 331;

(l) a polynucleotide encoding a chimeric polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:5;

(m) a polynucleotide comprising a nucleotide sequence complementary to any one of the polynucleotides specified in (a)-(l) above; and

(n) a polynucleotide capable of simultaneously hybridizing under stringent conditions to sequences encoding the chemokine polypeptide and to sequences encoding the heterologous polypeptide in any one of the polynucleotides specified in (a)-(m) above.

16. The composition of claim 1 wherein the polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 16 to nucleotide 1226;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 85 to nucleotide 1226;

(c) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:8;

(d) a polynucleotide comprising the nucleotide sequence of the full-length protein-coding sequence of clone MPB-X deposited under accession number ATCC XXXXX;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein-coding sequence of clone MPB-X deposited under accession number ATCC XXXXX;

(f) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:7;

(g) a polynucleotide encoding a chimeric polypeptide comprising the amino acid sequence of SEQ ID NO:7 from amino acid 24 to amino acid 331;

(h) a polynucleotide encoding a chimeric polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:7;

(i) a polynucleotide comprising a nucleotide sequence complementary to any one of the polynucleotides specified in (a)-(h) above; and

(j) a polynucleotide capable of simultaneously hybridizing under stringent conditions to sequences encoding the chemokine polypeptide and to sequences encoding the heterologous polypeptide in any one of the polynucleotides specified in (a)-(i) above.

17. A composition of claim 1 wherein the polynucleotide is operably linked to an expression control sequence.

18. A host cell transformed with a composition of claim 17.

19. The host cell of claim 18, wherein the cell is a mammalian cell.

20. A process for producing a chimeric polypeptide, which comprises:

(a) growing a culture of the host cell of claim 18 in a suitable culture medium; and

(b) purifying the chimeric polypeptide from the culture.

21. A polypeptide produced according to the process of claim 20.
22. The polypeptide of claim 21 comprising a mature polypeptide.
23. A composition comprising a chimeric polypeptide, the chimeric polypeptide comprising at least one chemokine polypeptide covalently attached to at least one heterologous polypeptide.
24. The composition of claim 23 wherein the heterologous polypeptide is covalently attached to the amino terminus of the chemokine polypeptide.
25. The composition of claim 24 wherein the chimeric polypeptide comprises a linker polypeptide covalently attached to the heterologous polypeptide and the chemokine polypeptide.
26. The composition of claim 23 wherein the heterologous polypeptide is covalently attached to the carboxyl terminus of the chemokine polypeptide.
27. The composition of claim 26 wherein the chimeric polypeptide comprises a linker polypeptide covalently attached to the heterologous polypeptide and the chemokine polypeptide.
28. The composition of claim 23 wherein the chemokine polypeptide is derived from SDF-1 $\alpha$ .
29. The composition of claim 28 wherein the chemokine polypeptide is SDF-1 $\alpha$ .
30. The composition of claim 23 wherein the chemokine polypeptide is derived from MIP-1 $\alpha$ .

31. The composition of claim 30 wherein the chemokine polypeptide is MIP-1 $\alpha$ .

32. The composition of claim 23 wherein the chemokine polypeptide is derived from MIP-1 $\beta$ .

33. The composition of claim 32 wherein the chemokine polypeptide is MIP-1 $\beta$ .

34. The composition of claim 23 wherein the heterologous polypeptide comprises an Fc polypeptide.

35. The composition of claim 23 wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:1;
- (b) the amino acid sequence of SEQ ID NO:1 from amino acid 20 to amino acid 328;
- (b) the amino acid sequence of SEQ ID NO:1 from amino acid 21 to amino acid 328;
- (c) the amino acid sequence of SEQ ID NO:1 from amino acid 22 to amino acid 328; and
- (d) fragments of the amino acid sequence of SEQ ID NO:1.

36. The composition of claim 23 wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:3;
- (b) the amino acid sequence of SEQ ID NO:3 from amino acid 20 to amino acid 326; and
- (c) fragments of the amino acid sequence of SEQ ID NO:3.

37. The composition of claim 23 wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:5;
- (b) the amino acid sequence of SEQ ID NO:5 from amino acid 23 to amino acid 331; and
- (c) fragments of the amino acid sequence of SEQ ID NO:5.

38. The composition of claim 23 wherein the chimeric polypeptide comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:7;
- (b) the amino acid sequence of SEQ ID NO:7 from amino acid 24 to amino acid 331; and
- (c) fragments of the amino acid sequence of SEQ ID NO:7.

39. The composition of claim 23 wherein the chimeric polypeptide comprises the amino acid sequence of SEQ ID NO:1.

40. The composition of claim 23 wherein the chimeric polypeptide comprises the amino acid sequence of SEQ ID NO:3.

41. The composition of claim 23 wherein the chimeric polypeptide comprises the amino acid sequence of SEQ ID NO:5.

42. The composition of claim 23 wherein the chimeric polypeptide comprises the amino acid sequence of SEQ ID NO:7.

43. The composition of claim 23, further comprising a pharmaceutically acceptable carrier.

44. A composition comprising an antibody which reacts with both the chemokine polypeptide and the heterologous polypeptide of claim 23.



45. A method for identifying molecules capable of interacting with a chimeric polypeptide which comprises:

- (a) combining a composition of claim 23 with a composition comprising molecules to be tested for interaction, forming a first mixture;
- (b) combining the first mixture with a composition comprising indicator molecules, so that the indicator molecules are capable of being altered by the first mixture; and
- (c) detecting the presence of altered indicator molecules.

46. A method for attracting migratory cells to a region of an organism which comprises administering therapeutically effective amounts of at least one composition of claim 23.

47. A method for stimulating angiogenesis which comprises administering therapeutically effective amounts of at least one composition of claim 23.

48. A method for inhibiting angiogenesis which comprises administering therapeutically effective amounts of at least one composition of claim 23.

49. A method for preventing, treating, or ameliorating an inflammatory condition which comprises administering therapeutically effective amounts of at least one composition of claim 23.

50. A method for preventing, treating, or ameliorating an autoimmune condition which comprises administering therapeutically effective amounts of at least one composition of claim 23.

51. A method for enhancing antigen-presenting cell activity which comprises administering therapeutically effective amounts of at least one composition of claim 23, wherein at least one chimeric polypeptide of claim 23 comprises antigen molecules.

52. A method for inducing an immune response which comprises administering a vaccine and therapeutically effective amounts of at least one composition of claim 23.

53. A method for altering receptor function which comprises causing a receptor to bind at least one chimeric polypeptide of claim 23.

54. A method for decreasing receptor function which comprises causing a receptor to bind at least one chimeric polypeptide of claim 23, resulting in a decrease in the number of functional receptor molecules.

55. A method for preventing, treating, or ameliorating HIV infection which comprises administering therapeutically effective amounts of at least one composition of claim 23.

56. A method of claim 55, wherein the compositions administered comprise a chimeric polypeptide of claim 23 comprising SDF-1 $\alpha$  and a chimeric polypeptide of claim 23 comprising a chemokine selected from the group consisting of MIP-1 $\alpha$  and MIP-1 $\beta$ .

Figure 1B

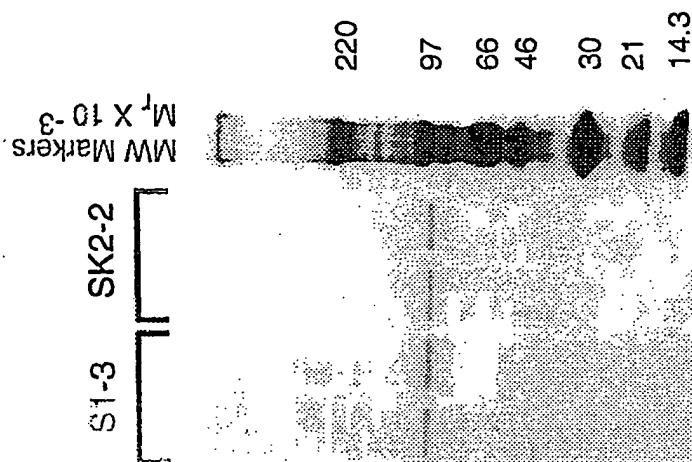


Figure 1A

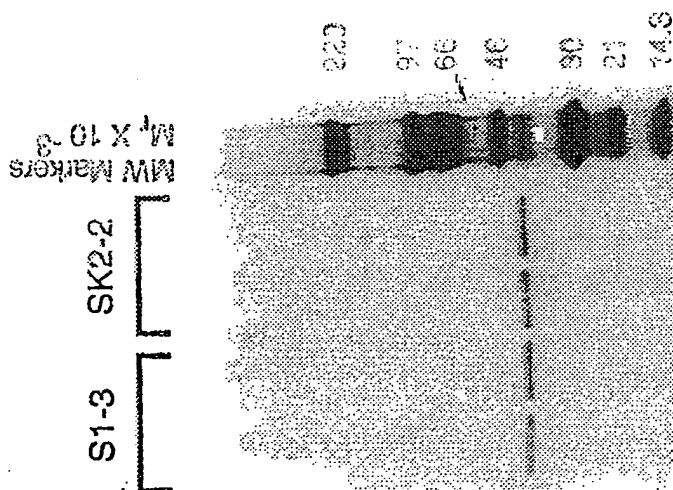


Figure 1D

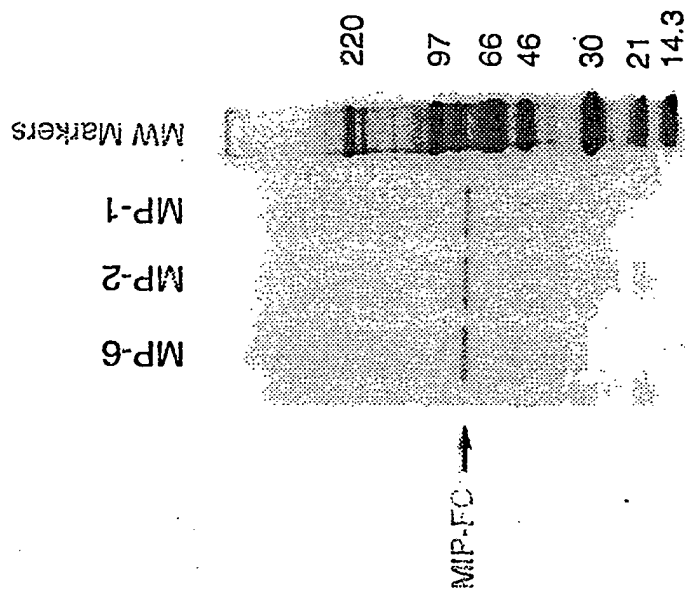
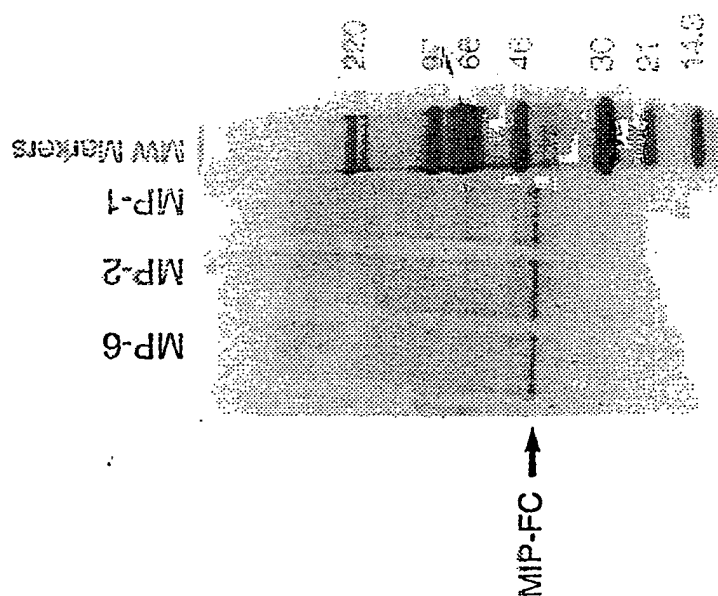
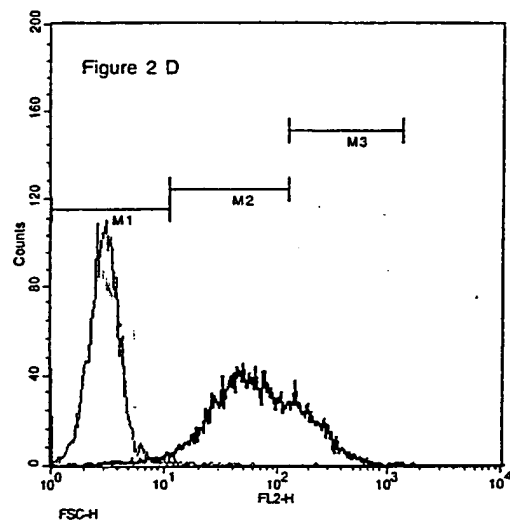
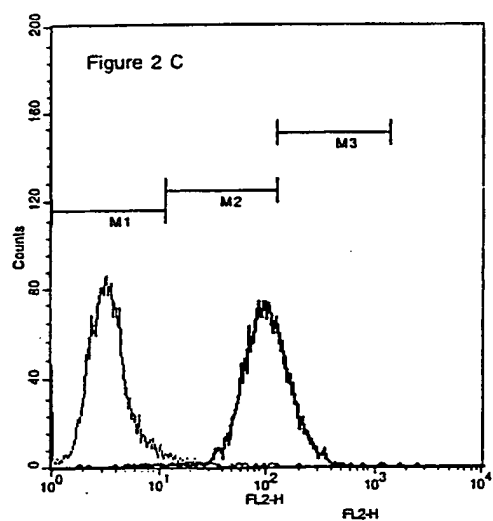
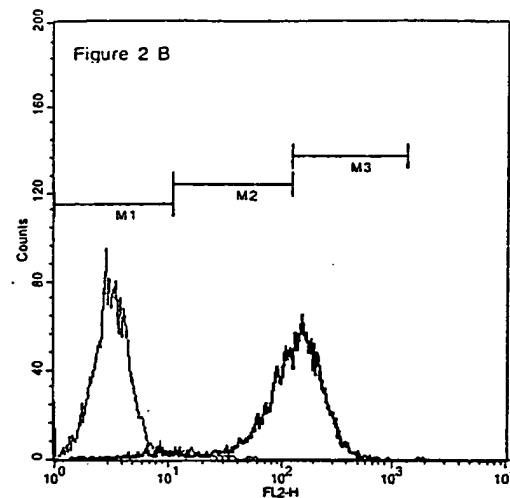
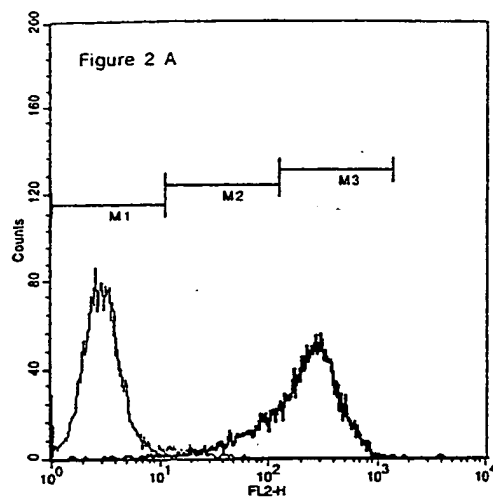


Figure 1C



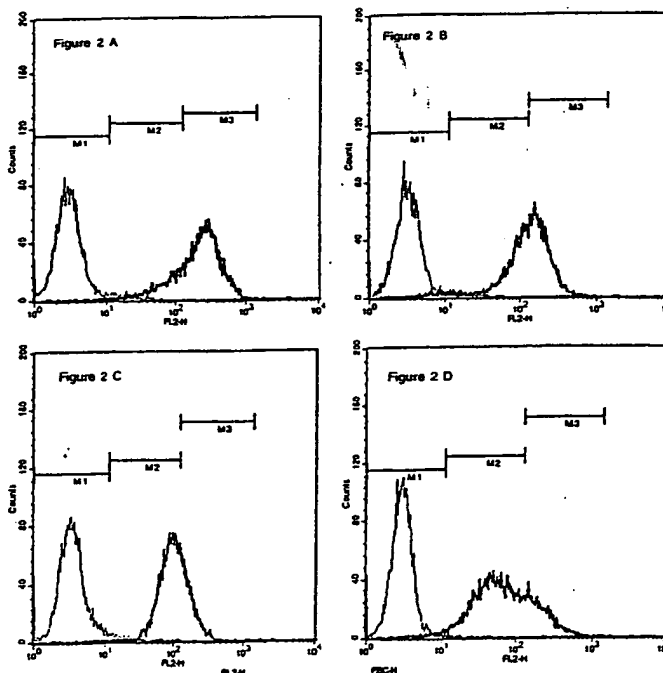




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : C12N 15/62, 15/19, C07K 14/52, 16/24, A61K 38/19, G01N 33/68, C12N 5/10, C07K 16/00		A3	(11) International Publication Number: <b>WO 98/38212</b>
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(21) International Application Number: PCT/US98/04002		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
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(71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US).			
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(74) Agent: SPRUNGER, Suzanne, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).		(88) Date of publication of the international search report: 14 January 1999 (14.01.99)	

## (54) Title: CHIMERIC POLYPEPTIDES CONTAINING CHEMOKINE DOMAINS



## (57) Abstract

This invention provides a chimeric DNA molecule comprising a sequence encoding a chemokine polypeptide covalently attached to a heterologous polypeptide, the encoded chimeric polypeptide, and uses thereof.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/04002

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/62 C12N15/19 C07K14/52 C07K16/24 A61K38/19  
G01N33/68 C12N5/10 C07K16/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TASHIRO K ET AL: "SIGNAL SEQUENCE TRAP: A CLONING STRATEGY FOR SECRETED PROTEINS AND TYPE I MEMBRANE PROTEINS" SCIENCE, vol. 261, 30 July 1993, pages 600-603, XP000673204 see the whole document ---	1,4,6,7, 13,14, 17-23, 26,28, 29,35, 36,45
X	WO 96 23888 A (SEARLE & CO ;STATEN NICHOLAS R (US); FAVARA JEAN P (US); KAHN LARR) 8 August 1996 see abstract; claims 15,16,23; example 19 --- -/--	1-5, 17-27, 43,44

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

13 August 1998

Date of mailing of the international search report

17.11.1998

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/04002

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 699 766 A (SQUIBB BRISTOL MYERS CO) 6 March 1996	1,4,5, 17-23, 26,27, 43,52
Y	see abstract; claims 1-6; figures 2-5,7 see page 8, paragraph 1 - page 9, paragraph 1 ---	12,34
X	WO 96 04388 A (SMITHKLINE BEECHAM PLC ;SMITHKLINE BEECHAM CORP (US); BROWNE MICHA) 15 February 1996	13,14, 35,36
Y	see abstract; claims 1,7,9-11,13-19; examples 1-4 ---	12,34
A	US 5 563 048 A (HONJO TASUKU ET AL) 8 October 1996 see abstract; claims 1-6; examples 7,9 ---	
A	HOWARD O M Z ET AL: "CHEMOKINES: PROGRESS TOWARD IDENTIFYING MOLECULAR TARGETS FOR THERAPEUTIC AGENTS" TRENDS IN BIOTECHNOLOGY, vol. 14, no. 2, February 1996, pages 46-51, XP002025857 see abstract; figure 1; table 1 see page 49 -----	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Int'l application No.

PCT/US 98/04002

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
See Further Information sheet enclosed
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

See extra sheet, subject 1.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-5, 12, 17-27, 34, 43-56 all partially and 6, 7, 13, 14, 28, 29, 35, 36, 39, 40 all complete

Compositions comprising SDF-1alpha and SDF-1alpha-derived fusion proteins, corresponding nucleic acids, host cells containing said nucleic acids, antibodies directed against said fusion proteins.

2. Claims: 1-5, 12, 17-27, 34, 43-56 all partially and 8, 9, 15, 30, 31, 37, 41 all complete

same as invention 1 but comprising MIP-1alpha and MIP-1alpha derived fusion proteins.

3. Claims: 1-5, 12, 17-27, 34, 43-56 all partially and 10, 11, 16, 32, 33, 38, 42 all complete

same as invention 1 but comprising MIP-1beta and MIP-1beta derived fusion proteins.

**REMARK:**

Although claims 46-56 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

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## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/04002

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9623888 A	08-08-96	AU 4658596 A CA 2212006 A EP 0807181 A	21-08-96 08-08-96 19-11-97
EP 0699766 A	06-03-96	US 5314995 A AT 166232 T CA 2034741 A DE 69129421 D EP 0439095 A ES 2115596 T JP 6087898 A US 5645835 A	24-05-94 15-06-98 23-07-91 25-06-98 31-07-91 01-07-98 29-03-94 08-07-97
WO 9604388 A	15-02-96	AU 3382595 A BR 9508469 A CA 2196200 A CN 1164872 A CZ 9700256 A EP 0770135 A HU 76369 A JP 10503371 T NO 970374 A PL 318380 A US 5783181 A	04-03-96 16-09-97 15-02-96 12-11-97 17-09-97 02-05-97 28-08-97 31-03-98 19-02-97 09-06-97 21-07-98
US 5563048 A	08-10-96	CA 2117953 A CN 1110323 A EP 0657468 A JP 7188294 A US 5756084 A	15-04-95 18-10-95 14-06-95 25-07-95 26-05-98

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